（H30-化学−一般-001）厚生労働科学研究費補助金（化学リスク研究事業）

令和元年度総括研究年度終了報告書

化学物質の動物個体レベルの免疫毒性データ集積とそれに基づくMulti-ImmunoTox assay（MITA）による予測性試験法の確立と国際標準化

（30210101）

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研究要旨

本課題においては，これまでに1)我々が開発した多項目免疫毒性評価系Multi-ImmunoTox Assay (MITA)の免疫毒性化学物質評価法としてのOECD テストガイドライン化に向けて国際validation試験ならびに2)免疫毒性化学物質のデータベース作成を行ってきた。1）においては，既にMITA を構成する試験法の一つである IL-2 Luc assay に関してvalidation試験を終了し，それに基づきvalidation report を作成しpeer review panelの評価を受けている。またIL-1 Luc assayに関してもphase I，phase IIのvalidation試験を終了し，2020年1月に行われる海外からのliaison委員を交えたvalidation management team (VMT)会議にて予測性を除いた試験結果の評価がなされる施設内施設間再現生結果が承認された。一方，2）においては，上記validation 試験にて評価した50化学物質，validation report作成にあたりMITAにて評価した60化学物質に関して免疫毒性データを収集し免疫毒性データベースを構築した。またMITAのOECDテストガイドライン申請に向けて，in vitro免疫毒性試験法の現状とMITAの有用性に関してdetailed review paperを作成しOECDに提出する準備を始めた。

研究分担者氏名・所属研究機関名及び所属研究機関における職名
小島　肇･国立医薬品食品衛生研究所安全性生物試験研究センター薬理部･室長
中島　芳浩･国立研究開発法人産業技術総合研究所･健康工学研究部門･研究グループ長
安野理恵･国立研究開発法人産業技術総合研究所･バイオメディカル研究部門･主任研究員
大森　崇･神戸大学医学部附属病院･臨床研究推進センター、生物統計学第二室長
木村　裕･東北大学病院･皮膚科･助教

A．研究目的

研究背景:

アレルギー、自己免疫、免疫抑制など、人体に有害な影響を及ぼす化学物質による免疫毒性は、消費者、生産者はもとより厚生労働行政にとっても重大な課題となっている。現在、免疫毒性評価のゴールドスタンダードは動物実験であるが、数万ともいわれる化学物質を網羅的に評価、管理するには、*in vitro* high throughput 評価系や *in silico* 評価系の構築が不可欠である。そのためには、化学物質のアレルギー発症、易感染性など個体レベルの免疫毒性データの集積、その分子メカニズムの解析、さらにはそれらに基づいた adverse outcome pathway の作成が不可欠である。

我々は，平成18–22年NEDO「高機能簡易型有害性評価手法の開発」プロジェクトにおいて、化学物質の免疫毒性多項目評価システム(Multi-ImmunoToxicity assay;MITA)を構築し国内外の特許を取得している。

また平成24年度から平成26年度の3年間にわたる厚生労働科学研究費補助金事業「多色発光細胞を用いたhigh-throughput免疫毒性評価試験法の開発」においては、作用機序の明らかな種々の免疫抑制剤をMITAにより評価するなかで、化学物質免疫毒性評価におけるMITAのプロトコールを作成し、そのプロトコールに基づいて薬剤の免疫毒性評価を行った。その結果、代表的な免疫抑制剤であるデキサメサゾン(Dex)、サイクロスポリン(CyA)、タクロリムス（Tac）のT細胞とマクロファージ/樹状細胞に対する薬理効果をMITAが予測できることを明らかにした[1,2]。

さらに平成27年度以降は、皮膚感作性試験法IL-8 Luc assayとMITAを組み合わせたmodified MITAを構築し60種類の化学物質を評価しdata setを作成した。また、そのdata setを基に化学物質のclusteringを行い、化学物質が免疫毒性のprofileの違いにより6つのグループに分類できることを示した[3]。さらに、研究期間中にIL-8 Luc assayをOECDテストガイドライン化することができた(OECD442E)[4,5]。

計画全体の目的:

1）既に OECD テストガイドライン(442E)に承認されている IL-8 Luc assay に加え、MITA を構成する IL-2 転写活性抑制評価試験(IL-2 Luciferase reporter assay; IL-2 Luc assay)と IL-β 転写活性抑制評価試験(IL-1 luciferase reporter assay; IL-1 Luc assay)の国際 validation study を行い、MITA の多項目免疫毒性評価系として OECD テストガイドライン化を目指す。

2)National Toxicology Program (NTP)の Dori Germolec 博士とミラノ大学の Emanuela Corsini 博士の協力を仰ぎ、NTP ならびに European Centre for Ecotoxicology and Toxicology of Chemicals のデータベースおよび PubMed を利用した文献検索に基づき免疫毒性のデーターベースを構築する。

3)上記データベースに基づき, MITA（図２）を用いた化学物質の免疫毒性別クラスター分類における各クラスター免疫毒性の特性を明らかにする。

2019 年度

① IL-2転写活性抑制試験 (IL-2 Luc assay)に関する validation report に対する peer review panelによる評価とそれに対する対応
② IL-1β転写活性抑制試験(IL-1 Luc assay)に関するPhase I, Phase II validation試験とValidation management teamによる最終評価
③ IL-1 Luc assay, IL-2 Luc assay により多種類の化学物質を評価し data set を作成する。
④ 免疫毒性化学物質のデータベース作成

⑤ MITAによる免疫毒性clusteringの有用性の検討
⑥ MITA を用いた免疫毒性評価系国際化へ向けて，detailed review paper 作成を目的とした国際会議の開催

B. 研究方法

① IL-2 Luc assay validation reportに対するpeer review panelによるコメントとそれに対する対応

以下の会議を開催し，peer review panelからIL-2 Luc assay validation reportに対するコメントが提出され，それらに対応した。

1. 1st International peer review panel meeting on Multi-Immunotoxicity Test Assay (MITA))
2019年2月27-28日，品川
Peer review panel: Henk van Loveren, Haley LaNef Ford, Barbara Kaplan, Sang-Hyun Kim, Fujio Kayama, Takao Ashikaga, Xingchao Geng
参加者：Hajime Kojima, Yutaka Kimura, Setsuya Aiba
2. 2nd International peer review panel meeting on Multi-Immunotoxicity Test Assay (MITA)(Webex)
2019年10月1日（火）
Peer review panel: Henk van Loveren, Haley Neff-LaFord, Barbara Kaplan, Fujio Kayama, Takao Ashikaga
参加者：Hajime Kojima, Yutaka Kimura, Setsuya Aiba
3. 3rd International peer review panel meeting on Multi-Immunotoxicity Test Assay (MITA) (Webex)
2019年11月18日（月）
Peer review panel: Henk van Loveren, Haley Neff-LaFord, Barbara Kaplan, Lin Shi, Xingchao Geng, Fujio Kayama, Takao Ashikaga
参加者：Hajime Kojima, Yutaka Kimura, Setsuya Aiba

② IL-1 Luc assay Phase IならびにPhase II validation試験

Phase I試験においては，国際バリデーション実行委員会 (VMT)にて選定された５化学物質をコード化し、東北大学，産業技術総合研究所バイオメディカル研究部門，産業技術総合研究所工学研究部門の参加３施設においてMulti-ImmunoTox Assay protocol for TGCHAC-A4 ver. 008Eにのっとり各物質3回繰り返し1セットの試験を３セットと実施した。
Phase II試験においては，VMTにより選定された20化学物質をコード化し、東北大学，産業技術総合研究所バイオメディカル研究部門，産業技術総合研究所工学研究部門の参加3施設においてMulti-ImmunoTox Assay protocol for TGCHAC-A4 ver. 008Eにのっとり各物質3回繰り返し1セットを実施した。
また，validation試験を遂行にあたり以下のVMT会議を行った。

1. 2019年度第1回MITAバリデーション電話会議 （スカイプ）
2019年4月5日（金）9:30-11:00
参加者：大森、髙木、小島、足利、相場、木村
2. 2019年度第2回MITAバリデーション電話会議 （スカイプ）
2019年5月2日（木）10:00-12:00
参加者：大森、小島、安野、中島、相場、木村、藤村
3. Conference call for the MITA assay (Webex)
2019年6月26日（水）20:00-
参加者：Corsini, E., Roggen, E., Germolec, D., Inoue, T., Aiba, S., Kimura, Y., Omori, T., Kojima, H.
4.5th meeting for the MITA Validation study
2020年1月30日（水）10：00-17：00
2020年1月31日（金）10：00-13：00
参加者：Corsini, E., Germolec, D., Inoue, T., Aiba, S., Kimura, Y., Omori, T., Kojima, H., Yasuno, R., Nakajima, Y.

③ IL-2 Luc assay，IL-1 Luc assayのdata set作成

Validation試験で評価した化学物質以外の化学物質もIL-1 Luc assay、IL-2 Luc assayにて評価し，これらの試験法のdata setを作成した。

④ 免疫毒性物質データベースの作成

National Toxicology Program (NTP)の Dori Germolec 博士とミラノ大学の Emanuela Corsini 博士の協力を仰ぎ、NTP ならびに European Centre for Ecotoxicology and

Toxicology of Chemicals のデータベースおよび PubMed を利用した文献検索に基づき，validation 試験で用いた化学物質，data set に際して評価した化学物質を中心に免疫毒性データベースを構築した。

⑤ MITAによる免疫毒性clusteringの有用性の検討

一方、我々はこれまでに 60 種類の化学物質を MITA の複数項目に関して効果発現最低濃度(Lowest observed effect level；LOWEL)を基にクラスター分類することにより、免疫毒性物質が 6 種類のクラスターに分類できることを明らかにした[3]。そこで、さらに改訂された上記データベースを参考に MITA によりクラスター分類を再検討する。

⑥ MITA を用いた免疫毒性評価系国際化へ向けての国際評価会議の開催

皮膚感作性試験法を除いては，in vitro 免疫毒性試験法は OECD テストガイドラインに存在しない。そこで，OECD 免疫毒性試験評価者の in vitro 免疫毒性評価系の現状と MITA の有用性の理解の促進を図る目的で， in vitro 免疫毒性評価法に関する detailed review paper (DRP) の作成を計画し以下の会議を開催した。

1.1st call for DRP in vitro immunotoxicity (Webex)
2019 年 9 月 18 日（水）、20 時
Emanuela Corsini, Erwin Roggen, Dori Germolec, Henk van Loveren, Barbara Kaplan, Setsuya Aiba, Yutaka Kimura, Takayuki Yoshimoto, Hajime Kojima, Steve Venti

2. 2nd call for DRP in vitro immunotoxicity (Webex)
2019 年 10 月 28 日（水）、20 時
Emanuela Corsini, Erwin Roggen, Dori Germolec, Henk van Loveren, Barbara Kaplan, Setsuya Aiba, Yutaka Kimura, Takayuki Yoshimoto, Hajime Kojima, Steve Venti

3.3rd meeting for OECD DRP on in vitro immunotoxicity.
2020 年 1 月 28 日 9:00-17:30
2020 年 1 月 29 日 9:00-15:00
Emanuela Corsini, Dori Germolec, Henk van Loveren, Barbara Kaplan, Setsuya Aiba, Yutaka Kimura, Takayuki Yoshimoto, Hajime Kojima, Steve Venti

（倫理面への配慮）

健常人からの採血に際しては、研究内容、採血における危険性、得られた検査結果により本人の人権が損なわれることのないこと、得られた検査結果は守秘され個人のプライバシーを侵害する可能性がないこと、研究に協力することに同意した後もいつでも自由に辞退できること、この研究によって生じる知的財産権は被験者には帰属しないことについて説明し、本人より同意書を取得している。

C. 研究結果

①IL-2 Luc assay validation reportに対するpeer review panelによるコメントとそれに対する対応

今回IL-2 Luc assay validation reportを作成するにあたり，施設内，施設間再現生は試験開始前の目標値であった80%を達成した。しかし予測性に関しては，そもそも医薬品を除く多くの化学物質の免疫毒性評価が必ずしも定まっていないため確定できないでいた。またpeer review pane会議にて，IL-2 Luc assayは免疫毒性一般を評価する試験系ではなく，T細胞を一次標的として免疫毒性を惹起する免疫毒性物質の評価系であり，それを加味して予測性を決定するように指導された。そこで，本試験において，NTPのLusterら[6-9]が51種類の化学物質の免疫毒性を動物実験を用いて評価した際の判定基準を参考にT細胞を標的とした化学物質の免疫毒性を評価する分類法を提案し，peer review panelにより了承された。分類方法は添付資料１を参照。これによりIL-2 Luc assayの予測性が決定した（添付資料２）。それに基づきvalidation reportを作成し提出した（添付資料３抜粋）。

我々が提出したvalidation reportに対して，1st International peer review panel meeting on Multi-Immunotoxicity Test Assay (MITA))にて添付資料4のaction items（簡略版）が提案された。それに対して，添付資料5で対応した。さらに我々の回答に対して，2nd International peer review panel meeting on Multi-Immunotoxicity Test Assay (MITA) (Webex)で

は，添付資料6のaction itemsが提案され，それに対して添付資料7で対応した。
更に，3rd International peer review panel meeting on Multi-Immunotoxicity Test Assay (MITA) (Webex)では，添付資料8のaction itemsが提案され，それに対して添付資料8の赤字にて回答した。

② IL-1 Luc assay Phase IならびにPhase II validation試験

IL-1 Luc assay Phase I試験を実施した。添付資料９に結果を示すが，within laboratory reproducibility, between laboratory reproducibility いずれも100%と極めて良好な結果が得られた。この結果に関して以下の会議を開催した。
2019年度第1回MITAバリデーション電話会議 （スカイプ）
2019年4月5日（金）9:30-11:00
参加者：大森、髙木、小島、足利、相場、木村

2019年度第2回MITAバリデーション電話会議 （スカイプ）
2019年5月2日（木）10:00-12:00
参加者：大森、小島、安野、中島、相場、木村、藤村

第１回VMT会議 Conference call for the MITA assay (Webex)
2019年6月26日（水））20:00-
参加者：Corsini, E., Roggen, E., Germolec, D., Inoue, T., Aiba, S., Kimura, Y., Omori, T., Kojima, H.

以上の会議で，予測性に関しての最終評価は定まっていないが，さらに20化学物質を用いて施設間再現性を評価するPhase II 試験を行う事が了承された。そこで，3施設でPhase II 試験を実施し2019年12月までに全ての施設が試験を完了した。そこで以下の会議で試験結果が検討された。その結果，施設間再現性はPhase II 試験のみの結果で80%（資料10），Phase I，II 試験を統合した結果で84%となり，Phase Iの施設間再現性と共に試験開始前に想定していた採択基準をクリーアした。しかし，IL-1 Luc assayの再現性に関しては更に議論が必要と言うことになり，最終結論は次回のMVT会議に持ち越された。
第２回VMT会議
2020年1月31日（水））
会場：国立医薬品食品衛生研究所
参加者：Corsini, E., Roggen, E., Germolec, D., Inoue, T., Aiba, S., Kimura, Y., Omori, T., Kojima, H.

③IL-1 Luc assay，IL-2 Luc assayのdata set作成

IL-1 Luc assay，IL-2 Luc assayおよびIL-8 Luc assay に関して，それぞれの試験法の最終判定基準に則りdata setを作成した(添付資料11)

④ 免疫毒性物質データベースの作成

IL-2 Luc assayのvalidationに用いた25化学物質，IL-2 Luc assayのdata set作成に用いた化学物質に関して免疫毒性データベースを作成した。（添付資料12，添付資料13）データベースでは，化学物質の毒性データをin vivo、ex vivo、in vitroデータの3種類に分類した。具体的には、in vivo データの中には、免疫臓器の重量変化，遅延型過敏症，易感染性，移植腫瘍に対する抵抗性が、ex vivo データには、化学物質を投与された個体から採取した免疫担当細胞を用いてin vitroで化学物質の影響を評価するサイトカイン産生試験，T細胞依存性性抗体産生試験 (T-cell dependent antibody response; TDAR)が、in vitroデータには、個体から採取した免疫担当細胞に、in vitroで化学物質を加えてそのサイトカイン産生能の変化を評価するサイトカイン産生試験，T細胞の増殖能を評価する細胞増殖試験などを含めた。この作成に当たっては，National Toxicology Program (NTP)の協力を仰いだ。

⑤ MITAによる免疫毒性clusteringの有用性の検討

あらたに得られたデータセットをもとに IL-8 Luc assay と組み合わせた MITA により化学物質の clustering を実施した。その結果を添付資料13 に示す。しかし，IL-1 Luc assay，IL-2 Luc assay，IL-8 Luc assay の組み合わせでは，以前論文で報告した IL-2 Luc assay，IL-8 promoter assay，IL-8 Luc assay の組み合わせで行ったようには綺麗に clustering できなかった。また残念ながら、MITA では、一部の DNA 合成、細胞増殖抑制機序に基づく免疫毒性物質が評価できないことも明らかになった。

⑥ MITA を用いた免疫毒性評価系国際化へ向けての国際評価会議の開催

MITA のテストガイドライン化に向けて in vitro 免疫毒性評価法に関する detailed review paper (DRP)の作成を計画し以下の会議を開催した。

1.1st call for DRP in vitro immunotoxicity (Webex)
2019 年 9 月 18 日（水）、20 時
2. 2nd call for DRP in vitro immunotoxicity (Webex)
2019 年 10 月 28 日（水）、20 時
Emanuela Corsini, Erwin Roggen, Dori Germolec, Henk van Loveren, Barbara Kaplan, Setsuya Aiba, Yutaka Kimura, Takayuki Yoshimoto, Hajime Kojima, Steve Venti

上記会議において，以下の様な項目と執筆担当者が決定した添付資料 14。さらに下記の会議にて draft 案が提案され，それの修正を行った。修正後の draft を添付する（資料 15）

3.3rd meeting for OECD DRP on in vitro immunotoxicity.
2020 年 1 月 28 日 9:00-17:30
2020 年 1 月 29 日 9:00-15:00
Emanuela Corsini, Dori Germolec, Henk van Loveren, Barbara Kaplan, Setsuya Aiba, Yutaka Kimura, Takayuki Yoshimoto, Hajime Kojima, Steve Venti

E. 考察

臨床的に使われる免疫抑制剤を除くと，化学物質の免疫毒性，特にヒトに対する免疫毒性の評価は定まっていない。確かに，個々の化学物質に関して，幾つかの免疫毒性評価試験を行った報告は多数存在するが，それらを総括して化学物質の免疫毒性の有無を総括した報告は我々が調べた限り存在しない。この問題は，免疫毒性試験法のvalidation試験を行う際に大きな障害となった。

そこで本課題において，化学物質の免疫毒性に関する文献資料を基に免疫毒性の有無を判定するクライテリアを提案した。幸い，本課題においてはvalidation試験と並行して行ってきた免疫毒性データベースが存在し，それをもとに分類することを検討した。その際に，Lusterら[6-9]が報告した免疫毒性分類法を参考にした。この方法では，51種類の化学物質をマウスに投与し，その動物を種々の免疫毒性試験法で評価し免疫毒性の有無を判定するクライテリアを提案している。またそのクライテリアの判定結果とマウス感染実験から得られた易感染性の有無との相関も検討している。IL-2 Luc assayの予測性の評価においても，ほぼLusterらのクライテリアを参考に，作成した化学物質免疫毒性データベースをもとに評価化学物質の免疫毒性の有無を決定した。この妥当性は，peer review panelにからも承認された。この評価法に基づくと，Phase I、IIをまとめたpredictivityは75%となった。以上の結果をもとにvalidation reportを提出し現在peer review panelからのコメントに対応している。

また，上記のようにIL-2 Luc assayのvalidation試験の予測性評価を通して本課題で作成した免疫毒性データベースの有用性が確認された。

IL-1 Luc assayに関しては，これまでに順調にPhase I ,Phase II試験を終了し，2020年1月に行われるVMT会議で良好な施設内，施設間再現性が評価され，現在予測性に関して検討中である。

最後に，IL-1 Luc assay，IL-2 Luc assayと免疫毒性評価法をOECDテストガイドライン化を進めるにあたり，detailed review paperを提出することにし既にOECDにSPSFを提出した。さらに，その中に含まれる項目と執筆担当者を決定した。さらに2020年1月において，draft案が提案され，それの修正を行った。修正後のdraftを添付する担当者が一同に介する会議を東京にて開催予定である。

一方，本課題のもう一つのテーマである化学物質の免疫毒性データベースの作成をNTPの協力を得て行った。25種類の化学物質の入手可能な免疫毒性データを網羅し、それらをin vivo，ex vivo、in vitroデータに分類し、さらにそれらを添付資料11,12にまとめた。その結果、各化学物質の大凡の免疫毒性profileが俯瞰可能となった。

IL-2 Luc assayのpredictivityに関しては、2019年2月27日から28日まで、東京にて開催予定のMITAのOECDガイドライン化に向けての国際評価会議にて検討する予定である。

E. 結論

本課題においては，これまで我々が開発した多項目免疫毒性評価系 Multi-ImmunoTox Assay (MITA)の免疫毒性化学物質評価法としての OECD テストガイドライン化に向けて国際的 validation 試験を行ってきた。2019 年度までに MITA を構成する試験法の一つである IL-2 Luc assay に関しては validation 試験を終了し，それに基づき validation report を作成し peer review panel の評価を受けている。また IL-1 Luc assay に関しても phase I，phase II の validation 試験を終了し，2020 年 1 月に行われる海外からの liaison 委員を交えた validation management team (VMT)会議にて施設内施設間再現生結果は承認された。また MITA の OECD テストガイドライン化にむけて，最終年度に提出する予定の in vitro 免疫毒性試験に関する detailed review paper の standard project submission form (SPSF)を提出した。また validation 試験において評価した化学物質，MITA の data set の中に含まれる化学物質に関して，既知の免疫毒性特性を文献的に収集し、本課題のもう一つのテーマである化学物質免疫毒性データベースの構築を進めた。

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F.研究発表

1．論文発表

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2．学会発表

1. 15th International Congress of Toxicology, Hawaii convention center, July 15, 2019. Immunotoxicological Profiling of Chemicals Using Novel In Vitro Assays. Setsuya Aiba

2. 木村裕他：Multi-ImmunoTox Assay (MITA) の予測性評価に必要な文献に基づく化学物質免疫毒性分類の試み 日本動物実験代替法学会 第 32 回大会（つくば）2019 年 11 月

H. 知的財産権の出願・登録状況（予定を含む。）

1．特許取得

1. 相場節也　齋藤るみ子　木村裕　近江谷克裕　中島芳浩　西井重明　山崎友実　安田真琴；特許第 5999644 号(2016)；多色発光細胞を用いた免疫毒性評価システム
2. 相場節也　木村裕　近江谷克裕　西井重明；特開 2014-3939；免疫毒性評価細胞を用いたＴＮＦ－α 阻害活性を定量化するシステム
3. 木村裕　相場節也；特開 2016-208851；ＴＬＲ刺激物質の検出方法

添付資料１．化学物質免疫毒性評価基準 (Criteria to determine immunotoxicity of chemicals induced by directly targeting T cells) (IL-2 Luc assay validation report から抜粋)

To determine the performance of the IL-2 Luc assay, it is crucial to understand the immunotoxicological characteristics of the chemicals used in the study. Since the IL-2 Luc assay focuses on the effects of chemicals on IL-2 transcription by T cells, we attempted to classify the chemicals into two categories: (i) immunotoxic chemicals which target T cells (TTCs), which include chemicals that directly affect T cell viability, T cell proliferation or T cell function and (ii) others (NTTCs), which include chemicals that do not directly affect T cell viability, T cell proliferation or T cell function. In this assay, to define TTCs, we first surveyed the literature and collected the following six findings regarding each of the chemicals proposed for use in the study (Table 1). Using these six findings, we defined TTCs by the 4 criteria according to the rationale for classifying immunotoxic chemicals reported by Luster et al (Luster et al., 1992) (Table 2). Namely, if chemicals satisfy one of 4 criteria, they are considered as TTCs. Then, by comparing the results of the IL-2 Luc assay (positive or no effect) with the classification of the chemicals (TTC or NTTC), we calculated the accuracy, sensitivity and specificity of the IL-2 Luc assay in the validation study.

Table 1. The immunotoxicological data obtained from the literature

Findings	Information
Finding 1	Decreased thymus weight
Finding 2	Increased or decreased IL-2, IFN-g, IL-4 or other T cell-specific cytokine mRNA expression or protein production by T cells ex vivo.
Finding 3	Increased or decreased IL-2, IFN-g, IL-4 or other T cell-specific cytokine mRNA expression or protein production by T cells in vitro.
Finding 4	Suppressed T cell proliferation
Finding 5	Suppressed cytotoxic T cell response
Finding 6	The NTP data clearly indicate that one of the immunotoxic mechanism of chemicals are attributed to its effect on T cells.

Table 2. The criteria to classify immunotoxic chemicals by affecting T cells.

Criteria	Definition
Criterion 1	If chemicals are demonstrated to decrease thymus weight, one finding among Finding 2 to Finding 5
Criterion 2	There are multiple reports of Finding 2 or Finding 3.
Criterion 3	There are reports of increased or decreased mRNA expression or protein production in two or more cytokines for Finding 2 or Finding 3.
Criterion 4	The presence of the NTP data including Finding 6.

添付資料 2．IL-2 Luc assay バリデーション試験最終結果（IL-2 Luc assay validation report から抜粋）

Chemical	CAS	Lab.A	Lab.B	Lab.C	concordance	T cell targeting
Phase I						
Dibutyl phthalate	84-74-2	PPP	PPP	PPP	1	Yes
Hydrocortisone	50-23-7	PNN	PPP	PPN	0	Yes
Lead(II) acetate	6080-56-4	PPP	PPP	PPP	1	Yes
Nickel(II) sulfate	10101-97-0	PPP	PPP	PPP	1	Yes
DMDTC	137-30-4	NNN	NNN	NNN	1	No
Phase II						
2,4-Diaminotoluene	95-80-7	N	N	N	1	No
Benzo(a)pyrene	50-32-8	P	P	P	1	Yes
Cadmium chloride	10108-64-2	N	N	N	1	Yes
Dibromoacetic acid	631-64-1	P	P	N	0	Yes
Diethylstilbestol	56-53-1	P	P	P	1	Yes
Diphenylhydantoin	630-93-3	N	N	N	1	Yes
o-Benzyl-p-chorolophenol	120-32-1	P	P	P	1	No
Ethylene dibromide	106-93-4	N	N	N	1	Yes
Glycidol	556-52-5	P	P	P	1	No
Indomethacin	53-86-1	P	P	P	1	Yes
Isonicotinic Acid Hydrazide	54-85-3	P	N	P	0	Yes
Nitrobenzene	98-95-3	N	S	N	0	Undetermined
Urethane, Ethyl carbamate	51-79-6	P	P	P	1	Yes
Tributyltin chloride	1461-22-9	P	P	P	1	Yes
Perfluorooctanoic acid	335-67-1	P	P	P	1	Yes
Dichloracetic acid	79-43-6	P	P	P	1	Yes
Toluene	108-88-3	N	N	N	1	No
Acetonitril	75-05-8	N	N	N	1	No
Mannitol	69-65-8	N	N	N	1	No
Vanadium pentoxide	1314-62-1	N	N	N	1	No
o-Benzyl-p-chorolophenol	120-32-1	P	P	P	1	No

Within-laboratory reproducibilities (%)	**80 (4/5)**	**100 (5/5)**	**80 (4/5)**		
	Average 86.7 (13/15)				
Between-laboratory reproducibilities (%) (Based on majority for Phase I)				80 (20/25)	
Sensitivity (%)	75.0 (12/16)	75.0 (12/16)	75.0 (12/16)		
	Average 75.0 (36/48)				
Specificity (%)	75.0 (6/8)	75.0 (6/8)	75.0 (6/8)		
	Average 75.0 (18/24)				
Accuracy (%)	75.0 (18/24)	75.0 (18/24)	75.0 (18/24)		
	Average 75.0 (54/72)				

添付資料 3．IL-2 Luc assay バリデーションレポート （目次のみ抜粋）

Report on a Validation Study of the IL-2 Luc Assay for Evaluating the Potential Immunotoxic Effects of Chemicals on T-Cells

Validation Management Team

添付資料４．IL-2 Luc assay validation report に対する Peer review panel からのコメント (1)

201902

Action Items to peer reviewers for the validation report on the IL-2 Luc assay

Evaluation Criterion 1: A rationale for the test method should be available, including a description of the human health effect, a clear statement of scientific need, and regulatory application.

PRP Comment: Together with a new title, the rationale needs to be stated clearly to be T-cell targeting.

Evaluation Criterion 2: The toxicological mechanisms and the relationship between the test method endpoint(s) with the biological effect as well as the toxicity of interest should be addressed, describing limitations of the test method.

PRP Comment: Needs to focus on IL-2, including the limitations described in the meeting minutes. The introduction needs to focus solely on IL-2 and the IL-2 Luc Assay. Discussion about its part in MITA should be left until the discussion section.

Evaluation Criterion 3: A detailed test method protocol should be available

PRP Comment: The commercial availability of the #2H4 cell line needs to be described.

Evaluation Criterion 4: The within and between laboratory reproducibility of the test method should be demonstrated

PRP Comment: Acceptable

Evaluation Criterion 5: Demonstration of the test method's performance should be based on testing of representative, preferably coded reference chemicals

PRP Comment: We think only four or five negatives is not enough, so we suggest that some additional testing of negatives be performed.

Evaluation Criterion 6: Predictive capacity should be demonstrated using representative chemicals.

PRP Comment: Predictive capacity needs to be reassessed based on today's proposed definition of T-cell–targeting chemicals.

Evaluation Criterion 7: All data should adequately support the assessment of the validity of the test method for peer review.

PRP Comment: A clear definition of the 35% threshold and a clear explanation of Criteria 5 and how it was developed is needed. Should the table in Appendix 8 include the test judgment? Also, delete DTH, tumor, infection, and NK activity but specify T-cell proliferation in the table in Appendix 8.

Evaluation Criterion 8: All data from the validation study supporting the validity of a test method should be obtained in accordance with the principles of Good Laboratory Practice (GLP)

PRP Comment: The report needs to explain clearly and in detail what is meant by the phrase "in the spirit of GLP" and whether or not each laboratory performed their work in this spirit.

Evaluation Criterion 9: Applicability domain of the test method should be defined

PRP Comment: We recommend that the applicability domain be more clearly defined as noted in the PRP meeting minutes.

Evaluation Criterion 10: Proficiency chemicals should be set up in the proposed protocol

PRP Comment: None

Evaluation Criterion 11: Performance standards should be set up with the proposed protocol

PRP Comment: If performance standards are understood to be assay controls, then the use of three-fold stimulation of IL-2 Luc by PMA/IO and inhibition of stimulated IL-2 Luc by DEX and CYA are sufficient. We suggest that acceptance criteria for variability within test replicates be defined.

Evaluation Criterion 12: Advantages in terms of time, cost and animal welfare

PRP Comment: We suggest that the conclusion leave out mention of in vivo testing to confirm T-cell immunotoxicity and include discussion of the use of IL-2 Luc assay within MITA.

Evaluation Criterion 13: Completeness of all data and documents supporting the assessment of the validity of the test method.

PRP Comment: We suggest that data be redone to reassess predictive capacity based on today's proposed definition of T-cell–targeting chemicals. Also, a critical assessment of the 35% threshold in the context of the new definition of T-cell targeting is necessary.

Evaluation Criterion 14: Validation Study Management and Conduct

PRP Comment: None

Other considerations

PRP Comment: None

Conclusion

PRP Comment: We look forward to seeing a revised report based on our comments.

添付資料５． Peer review panel からのコメント (1)に対する対応

Dear the PRP:

Thank you for your kind and constructive comments and suggestions. We responded to each comment below and revised the VR taking the PRP comments into consideration. We used red fonts in the revised or newly added parts.

Evaluation Criterion 1: A rationale for the test method should be available, including a description of the human health effect, a clear statement of scientific need, and regulatory application.

PRP Comment: *Together with a new title, the rationale needs to be stated clearly to be T-cell targeting.*

The title was revised and changed to “Report on a Validation Study of the IL-2 Luc Assay for Evaluating the Potential Effect of Chemicals on T-Cells”.

The rationale to judge chemicals whether they were T-cell targeting or not was described in 10-3-1.

10-3-1. Rationale to determine the predictivity of the IL-2 Luc assay by the concordance between positive effects and the immunotoxic effects targeting T cell response

A well-functioning immune system is essential in maintaining the integrity of the organism. Therefore, immune dysregulation caused by chemicals, i.e., immunotoxic effects of chemicals, may make serious impacts on human health. It ranges from reduced resistance to infection and neoplasia to allergic and autoimmune conditions. The immune system comprises innate and adaptive immunity (Fig. 2). Both arms of the immune response function differently and are driven by different populations of cells. Chemicals can potentially affect the immune system by targeting either the innate immune system or the acquired immune system (Fig. 2 and Fig. 3). Therefore, *in vitro* test methods to detect immunotoxic effects of chemicals are needed to adequately assess their effects on both arms of immune system. However, it is impossible to predict the toxic effects of chemicals on the whole aspects of immune system by a single in vitro assay. Consequently, to accomplish the final goal of in vitro immunotoxicity tests that cover the whole aspects of immune system, it is indispensable to develop an integrated approach composed of multiple in vitro immunotoxic tests evaluating different aspects of immune responses. The MITA including the IL-2 Luc assay was developed to be components of the integrated approach.

Among various immune responses, one of pivotal responses is the development of antigen-specific effector T-helper subtypes, such as, Th1 cells, Th2 cells, Th17 cells, and regulatory T cells (Treg cells) that are associated with the clinical features and disease progression (reviewed by [1]). Therefore, the in vitro assay to clarify the effects

of chemicals on the development of these T-helper subtypes is one of the critical components of the integrated approach.

Now it is known that IL-2 exerts pleiotropic actions on CD4+ T cell differentiation via its modulation of cytokine receptor expression. It promotes Th1 differentiation by inducing IL-12Rb2 (and IL-12Rb1), promotes Th2 differentiation by inducing IL-4Ra, inhibits Th17 differentiation by inhibiting gp130 (and IL-6Ra), and drives Treg differentiation by inducing IL-2Ra. IL-2 also potently represses IL-7Ra, which decreases survival signals that normally promote cell survival and memory cell development (reviewed by [2]). Therefore, it is conceivable that chemicals, which affect IL-2 release by T cells, give significant impact on the development of Th cells.

When immunotoxic information of chemical is collected from the literature, however, most of the published data are not focusing on the effects of chemicals on the development of Th subsets. To overcome this problem, in this study, the predictivity was evaluated by the criteria whether chemicals affect T cell functions, namely T cell targeting, or not. To determine T cell targeting chemicals (TTCs), we collected the following 6 components in the literature.

#1. The decreased thymus weight

#2. The increased or decreased IL-2, IFN-g, or IL-4 mRNA expression or production by T cells in ex vivo.

#3. The increased or decreased IL-2, IFN-g, or IL-4 mRNA expression or production by T cells in vitro.

#4. The suppression of T cell proliferation

#5. The suppression of cytotoxic T cell response

#6. There is a clear statement in the NTP data that one of the immunotoxic mechanism of chemicals are attributed to its effect on T cells.

Then, we determined TTCs as chemicals that satisfied one of the following criteria

1) The combination of more than two components among #1 to #5 components
2) Multiple reports on #2 or #3
3) #2 or #3 on two or more cytokines
4) #5

Evaluation Criterion 2: The toxicological mechanisms and the relationship between the test method endpoint(s) with the biological effect as well as the toxicity of interest should be addressed, describing limitations of the test method.

PRP Comment: *Needs to focus on IL-2, including the limitations described in the meeting minutes. The introduction needs to focus solely on IL-2 and the IL-2 Luc Assay. Discussion about its part in MITA should be left until the discussion section.*

The limitation of this assay was described in the applicability domain (10-6).

10-6. Limitations and drawback, and applicability domain of the IL-2 Luc assay

Since the 2H4 cell line used in the IL-2 Luc assay is derived from Jurkat cells, it is conceivable that this cell line is more resistant to the cytotoxic effects of chemicals than bone marrow cells. Indeed, our study demonstrated that the IL-2 Luc assay cannot evaluate the immunotoxic effects of some immunosuppressive drugs which act by inhibiting DNA synthesis leading to myelotoxicity [3]. Thus, these chemicals in addition to chemicals that need metabolic activation should be outside the applicability domain. To overcome this drawback at present, the IL-2 Luc assay must be combined with assays capable of detecting myelotoxicity, such as the conventional 28-day subacute toxicity test [4] or *in vitro* myelotoxicity tests [5]. Similar to other *in vitro* test methods, poor water soluble chemicals are not suitable for this assay.

The introduction was revised according to the PRP comment. The detailed discussion on the MITA was moved to the Discussion.

Evaluation Criterion 3: A detailed test method protocol should be available

PRP Comment: The commercial availability of the #2H4 cell line needs to be described.

2H4 cells will be obtained from the GPC laboratory, Tottori, Japan after this assay is accepted as the test guideline.

Evaluation Criterion 4: The within and between laboratory reproducibility of the test method should be demonstrated

PRP Comment:*Acceptable*

Evaluation Criterion 5: Demonstration of the test method's performance should be based on testing of representative, preferably coded reference chemicals

PRP Comment: *We think only four or five negatives is not enough, so we suggest that some additional testing of negatives be performed.*

We reconsidered the immunotoxic characteristics of chemicals evaluated in Phase I and II studies. Finally, these two studies contained 7 negative chemicals (Appendix 8).

Evaluation Criterion 6: Predictive capacity should be demonstrated using representative chemicals.

PRP Comment: *Predictive capacity needs to be reassessed based on today's proposed definition of T-cell–targeting chemicals.*

We admit that it is crucial to more clearly define the criteria to classify chemicals into T cell-targeting chemical (TTC) and non-T cell-targeting chemical (NTTC). So, we proposed the new criteria with the international expert members, Dr. Emanuela Corsini and Dr. Dori Germolec taking PRP's proposal into consideration. The following was the revised session of predictivity (Revised VR 9-1-3).

9-1-3. Predictivity

To determine the predictivity of the IL-2 Luc assay, it is crucial to understand the immunotoxic characteristics of chemicals used in the study. Since the IL-2 Luc assay focuses on the effects of chemicals on IL-2 transcription by T cells, we tried to classify chemicals into those that affect T cell function, i.e., T cell-targeting chemical (TTC) and those that do not directly affect T cell function, i.e., non-T cell-targeting chemicals (NTTC). In this assay, to define TTCs, we collected the following 6 components in the literature.

#1. The decreased thymus weight

#2. The increased or decreased IL-2, IFN-g, or IL-4 mRNA expression or production by T cells in ex vivo.

#3. The increased or decreased IL-2, IFN-g, or IL-4 mRNA expression or production by T cells in vitro.

#4. The suppression of T cell proliferation

#5. The suppression of cytotoxic T cell response

#6. There is a clear statement in the NTP data that one of the immunotoxic mechanism of chemicals are attributed to its effect on T cells.

Then, we defined TTCs as chemicals that satisfy one of the following criteria

1) The combination of more than two components among #1 to #5 components
2) Multiple reports on #2 or #3
3) #2 or #3 on two or more cytokines
4) #5

To classify 25 chemicals used in the Phase I and II studies, we used the chemical information kindly provided by the National Toxicology Program (NTP). The immunotoxic characteristics of each chemical are shown in Appendix 7 and their summarized data are shown in Appendix 8. The table in Appendix 8 is the combined data of the NTP data and the data collected by the VMT member. As already described, IL-2 exerts pleiotropic actions on CD4+ T cell differentiation via its modulation of cytokine receptor expression. Indeed, IL-2 promotes Th1 and Th2 differentiation, while it also drives Treg differentiation. Therefore, it suggests that the augmentation of IL-2 transcription can lead to either immunostimulation or immunosuppression depending on surrounding tissue environment *in vivo.* Therefore, in this assay, if chemicals were judged as either augmentation or suppression, they were both considered as positive (P) and if not, they were judged as negative (N). Then we examined concordance between positive judgment and TTC.

Based on the new criterial for chemical classification, the predictivity of the Phase I and Phase II studies was summarized in 10-3-2.

10-3-2. The predictivity of the Phase I and Phase II studies

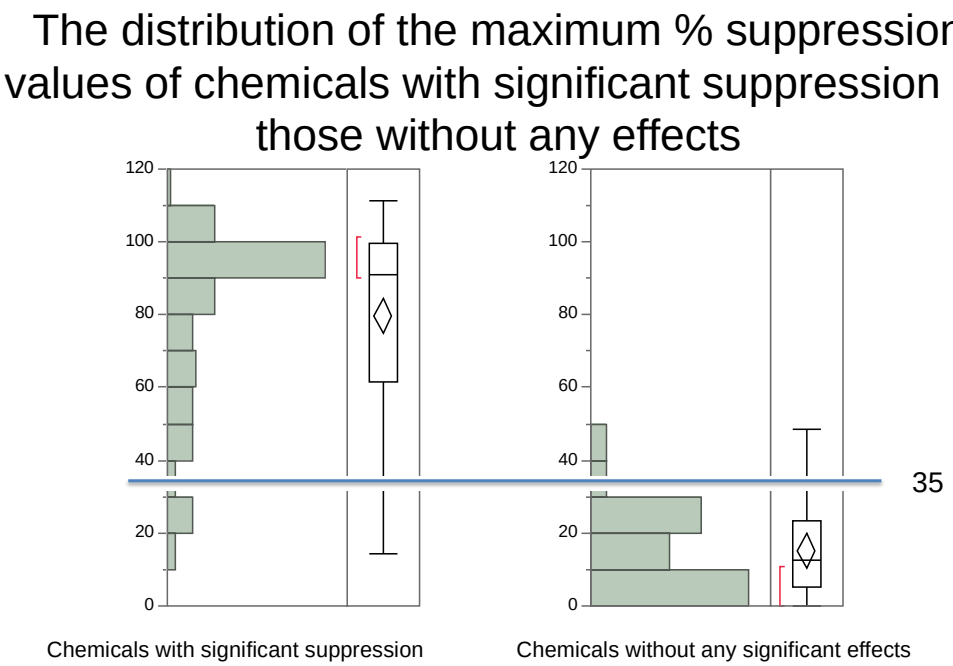
To classify 25 chemicals used in the Phase I and II studies, we used the chemical information kindly provided by the National Toxicology Program (NTP) and those collected by the VMT members. The immunotoxic characteristics of each chemical are shown in Appendix 7 and their summarized data are shown in Appendix 8. Based on the criteria, the 25 chemicals were classified into 14 TTCs, 9 NTTCs, and 2 unclassified chemicals that could not be classified because of insufficient data. According to this classification, the sensitivities of the assays as conducted by Lab A, Lab B, Lab C, and their average in the combined data of the Phase I and II studies are 80.0%, 80.0%, 73.3% and 77.7%, respectively. The specificities of the assays as conducted by Lab A, Lab B, Lab C, and their average are 75.0%, 75.0%, 75.0%, and 75.0%, respectively. The accuracies of the assays conducted by Lab A, Lab B, Lab C, and their average are 78.2%, 78.2%, 73.9%, and 76.8%, respectively.

Evaluation Criterion 7: All data should adequately support the assessment of the validity of the test method for peer review.

PRP Comment: *A clear definition of the 35% threshold and a clear explanation of Criteria 5 and how it was developed is needed. Should the table in Appendix 8*

include the test judgment? Also, delete DTH, tumor, infection, and NK activity but specify T-cell proliferation in the table in Appendix 8.

To determine the optimum threshold, we first potted the maximum % suppression values of chemicals with statistically significant suppression or those without any effects. The comparison of these two graphs showed that the threshold 35 can divide chemicals with significant suppression and those without any effects with minimum false positive or negative results.



The values of the maximum % suppression were derived from the data set made by the lead laboratory in our recent publication in Arch Toxicol (see the attached file)

We revised Appendix 8. As suggested, we deleted test judgment, DTH, infection, tumor rejection, and NK activity, and specified T cell proliferation.

Evaluation Criterion 8: All data from the validation study supporting the validity of a test method should be obtained in accordance with the principles of Good Laboratory Practice (GLP)

PRP Comment: The report needs to explain clearly and in detail what is meant by the phrase "in the spirit of GLP" and whether or not each laboratory performed their work in this spirit.

Evaluation Criterion 9: Applicability domain of the test method should be defined

PRP Comment: We recommend that the applicability domain be more clearly defined as noted in the PRP meeting minutes.

We described the applicability domain more precisely, taking the PRP comments into consideration in 10-6.

10-6. Limitations and drawback, and applicability domain of the IL-2 Luc assay

Since the 2H4 cell line used in the IL-2 Luc assay is derived from Jurkat cells, it is conceivable that this cell line is more resistant to the cytotoxic effects of chemicals than bone marrow cells. Indeed, our study demonstrated that the IL-2 Luc assay cannot evaluate the immunotoxic effects of some immunosuppressive drugs which act by inhibiting DNA synthesis leading to myelotoxicity [3]. Thus, these chemicals in addition to chemicals that need metabolic activation should be outside the applicability domain. To overcome this drawback at present, the IL-2 Luc assay must be combined with assays capable of detecting myelotoxicity, such as the conventional 28-day subacute toxicity test [4] or *in vitro* myelotoxicity tests [5]. Similar to other *in vitro* test methods, poor water soluble chemicals are not suitable for this assay.

Evaluation Criterion 10: Proficiency chemicals should be set up in the proposed protocol

PRP Comment:*None*

Evaluation Criterion 11: Performance standards should be set up with the proposed protocol

PRP Comment: *If performance standards are understood to be assay controls, then the use of three-fold stimulation of IL-2 Luc by PMA/IO and inhibition of stimulated IL-2 Luc by DEX and CYA are sufficient. We suggest that acceptance criteria for variability within test replicates be defined.*

Based on the PRP comments, we added the performance standard in the revised VR, Appendix 15.

Evaluation Criterion 12: Advantages in terms of time, cost and animal welfare

PRP Comment: *We suggest that the conclusion leave out mention of in vivo testing to confirm T-cell immunotoxicity and include discussion of the use of IL-2 Luc assay within MITA.*

In the revised VR, we deleted the description of requirement of in vivo testing. In addition, we described the potential of the IL-2 Luc assay (10-7).

10-7. Potential of the IL-2 Luc assay

The IL-2 Luc assay evaluates the effects of chemicals on IL-2 transcription by Jurkat T cells stimulated with PMA and CI. The simultaneous stimulation of PMA and calcium ionophore or ionomycin surrogates the stimulation by T cell receptor (TCR) and CD28 [6, 7]. The downstream signaling after the stimulation by TCR/CD28 is shown in Fig. 17. It indicates that the signaling required for IL-2 transcription after TCR/CD28 or PMA/CI stimulation involves the pathways leading the activation of AP1/2, mTOR, NF-kB, and NFAT. The immune system is composed of innate immune system and acquired immune system at least. The innate immune systems are activated by pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patters via Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), Nod-like receptors (NLRs), or cytokine receptors for IL-1 family or TNF family. Most of the downstream signaling after the stimulation of these receptors involves NF-kB and AP1/2 pathways [8]. In the acquired immune system, in addition to the process of T cell activation, B cell activation after B cell receptor stimulation and the signaling of various cytokines also involves NF-kB pathway (reviewed by Zhang and Sun [9]. Therefore, it is conceivable that the effects of chemicals on quite a few aspects of immune responses can be detected by the IL-2 Luc assay.

Evaluation Criterion 13: Completeness of all data and documents supporting the assessment of the validity of the test method.

PRP Comment: *We suggest that data be redone to reassess predictive capacity based on today's proposed definition of T-cell–targeting chemicals. Also, a critical assessment of the 35% threshold in the context of the new definition of T-cell targeting is necessary.*

In the revised validation report, we clearly defined the T cell-targeting chemicals. Based on the definition, we classified chemicals into T cell-targeting chemicals (TTCs) or non-T cell targeting chemicals (NTTCs). According to this classification, we recalculated the sensitivity, specificity, and accuracy of the Phase I and II studies.

Evaluation Criterion 14: Validation Study Management and Conduct

PRP Comment: None

Other considerations

PRP Comment: None

Conclusion

PRP Comment: We look forward to seeing a revised report based on our comments.

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添付資料 6．Peer review panel との teleconferance の議事録

Teleconference for IL-2 PRP

October 1, 2019

Peer Review Panel: Henk van Loveren, Haley Neff-LaFord, Barbara Kaplan, Fujio Kayama, Takao Ashikaga
VMT: Hajime Kojima
Observers: Steve Venti (meeting minutes)

Kojima:	In this meeting, we will discuss the revised validation report and the schedule going forward. I will explain the changes in the report, which are shown in red. One important point is Appendix 7. It has 290 pages and discusses the data available on immunotoxic effects of chemicals. Mainly, the figures for predictivity and the summary were revised. I heard Dr. Aiba is on-going to revise minorly. After the meeting, I ill share the newest Validation report.
Kaplan:	This summary is in line with what we discussed at the FTF meeting.
Kojima:	Does everyone accept this summary?
Everyone:	Yes.
Kojima:	Section 9-1-3 addresses predictivity and describes the effects of chemicals on T-cells. And there is a definition of T-cell targeting chemicals (TTCs).
Kaplan:	Criterion 3 says "#2 or #3 on two or more cytokines." Does that refer only to the three cytokines mentioned in #2 and #3? For example, is IL-17 excluded? This is not clear. If there is a report for other cytokines, would they be considered TTCs?
Kojima:	I can't answer at the moment, but I will ask Dr. Aiba.
Kaplan:	This is an improvement over the original report. Once we have some clarification on Criterion 3, I think that these criteria are acceptable.
van Loveren:	Although I think it would be good to extend this to other cytokines, not just the ones listed.
Kojima:	(Brief review of other changes in red. Please see revised Validation Study Report.) If you are happy with this report, then we can move on to reviewing the PRP Evaluation Criteria and creating the PRP report.
Kaplan:	Do we need to read this and provide comments? What do you need from the PRP to submit to the OECD?
Kojima:	If you feel that the Validation Study Report satisfies the 14 PRP Evaluation Criteria, then you can prepare a Peer Review Report of about 12 pages with a comment about each criterion. And then the Validation Study Report and the Peer Review Report will be reviewed by an OECD expert working group.
van Loveren:	Are there specific places we should comment on?
Kojima:	We revised the Validation Report based on the comments from the PRP.
Kaplan:	So we have already covered the critical issues. But if there is anything specific you want us to look at, please tell us now.
van Loveren:	Is there any issue we need to address now?
Kojima:	I will share these documents with you, and after we have your comments, Dr. Kayama will write the final PRP report.
Neff-LaFord:	Once you see the documents, it is pretty easy to follow what has been changed, so we should be able to follow it.
Kojima:	The deadline for comments if possible, would be by the end of October and then we can have another teleconference in early or mid-November. OK, I will send you meeting minutes, the newest validation Study Report, and the evaluation

添付資料７． Teleconferance のコメントに対する対応

October 4th, 2019

The response to the reviewers' comments:

Thank you for your kind consideration and important suggestions to the validation report. We revised the validation report according to the reviewers' comments. In addition, we corrected the values of the predictivity of this assay because there was one calculation error and we changed the classification of chemicals based on several references we found. The modified part was as follows. All the modified parts were written in red.

1. We modified the criteria to classify immunotoxic chemicals according to the reviewers' comments. (9-1-3. Predictivity in Page 61 and 10-3-1. Rationale to determine..... in Page 82)
2. We recalculated the predictivity. Consequently, the predictivity of the Phase II study, the combined Phase I and Phase II studies, and the data set was slightly changed. Briefly, the average predictivity of the Phase II was changed from 74.0% (40/54) to 70.2% (40/57). The average predictivity of the combined Phase I and Phase II studies was changed from 76.8% (53/69) to 75.0% (54/72). The predictivity of 60 chemicals was not changed. These changes were precisely described in Abstract, 9-4-3. Predictivity in the Phase II study, Table 22, 9-6-3. Predictivity in the Phases I and II studies, Table 23, 10-3-2. The predictivity of the Phase I and Phase II studies, 10-4. IL-2 Luc assay data set for 60 chemicals, and Table 24.
3. While revising the VR, we found a very crucial report by Luster et al, 1992. In their manuscript (Luster et al., 1992b), they proposed the rationale for immunotoxic classification. Namely, their proposal was that a positive was established on the basis that the test material either produced significant dose-response effect in the immune tests or significantly altered two or more test results at the highest dose of chemical tested. Furthermore, they classified chemicals based on their results of immune tests according to this rationale and found that there was a significant correlation between the judgment of immunotoxic chemicals and the host resistance (Luster et al., 1993). Therefore, we referred to their paper in 9-1-3. Predictivity and 10-3-1. Rationale to determine…..

4. We also added the comparison between the predictivity of the IL-2 Luc assay and that reported by Luster et al (Luster et al., 1992a; Luster et al., 1993; Luster et al., 1992b)and between the predictivity of the IL-2 Luc assay and that of the human whole blood cytokine release assay by Langezaal et al(Langezaal et al., 2002) in 10.4. IL-2 Luc assay data set for 60 chemicals.

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添付資料 8.

Teleconference for IL-2 PRP

November 11, 2019

Peer Review Panel: Henk van Loveren, Barbara Kaplan, Haley Neff-LaFord, Fujio Kayama, Takao Ashikaga, Lin Shi, Xingchao Geng
VMT: Hajime Kojima, Setsuya Aiba, Takuya Kimura
Observers: Steve Venti (meeting minutes)

Kojima:	In this meeting, we will discuss the revised validation report prior to discus items. We revised the report based on your comments. After the previous we received it in accordance with the comments from Barbara, and you hav comments that have not been reflected yet, so I think we need to discuss th
Kaplan:	I think these revisions are fine as long as things are separated into a table o intelligible.
Aiba:	I don't know who made this table, but it presents what I wanted to say, so I this if the PRP agrees.
Kojima:	Dr. Aiba will calculate predictive capacity based on this table, so the most i that the PRP finds this table acceptable.
Kayama:	I think these criteria are easier to understand as presented in the table.
van Loveren:	I am still concerned that the introduction is confusing to a naïve reader. We understand that MITA is the context, *not* the aim, of this study. But the intr clear statement at the start of the introduction that the aim of this validatio not MITA in general. Mentioning MITA in the introduction is fine, but you at the start of the introduction. The introduction must begin with the aim of IL-2. According to the reviewer's suggestion, I changed the abstract and began it this study.
Kaplan:	The first time I read this introduction, I thought that you were validating th later I realized that is not the case. The goal is to validate the IL-2 assay. I a Haley that the goal of the validation needs to be stated clearly at the start o Even just one sentence is enough. Just clearly state that the goal is to valida As described in the response to Dr. van Loveren's comment, I changed the it with the purpose of this study.
Neff-LaFord:	Yes, just more section 3 up higher. As suggested by the reviewer, we moved the objective of the study to secti
van Loveren:	We need to say "proposed AOP" because this AOP has not yet been accept As suggested, we added "proposed " in 3-9. The proposed Adverse Outcom of chemicals that affect IL-2 transcription.
Neff-LaFord:	The expression "IL-2 LA" appears to mean the same thing as "IL-2 Luc As intended to mean something different, then this needs to be spelled out mo According to the reviewer's comment, we modified Table 3. Definition of t

Aiba:	Yes, I will clarify that.
van Loveren:	On page seven in introduction, I have suggested a revision, but perhaps the the applicability range that I deleted needs to be added back.
Kaplan:	I think that in context, the meaning of “applicability domain” is clear enou the word “however” should be removed for clarity. As suggested by several reviewers, we deleted “however”.
van Loveren:	The applicability domain is discussed in the preceding paragraph, so mayb Haley’s suggestion as is.
Kojima:	In section 9-5, I will inform you the detailed records collected in the princi
Neff-LaFord:	I don’t understand what “almost comparable” means in section 10-3-1. We changed “comparable” to “similar to”, which is now in section 10-7.
Kaplan:	Given the emphasis on comparing IL-2 results with the results of other test section needs to be expressed more clearly. I think this information is impo it should be described more clearly. In the revised VL, we tried to describe more clearly the following sections,
Ashikaga:	I couldn’t find any description about regulatory application in the report. We added a new section describing the regulatory application (10-9)
Aiba:	Do I need to respond to each of these comments one by one?
Ashikaga:	Why is SFO-luciferase activity measured in this assay? We made a comment for the reason to ignore SLO-luciferase activity or IF
Aiba:	It is automatically measured but it is not necessary for this assay.
Kaplan:	This is related to what we were talking about before. This report contains a that is only incidentally related to IL-2, which confuses the reader.
Ashikaga:	I could not find a list of proficiency chemicals. Shouldn’t the developer sub
Aiba:	Yes. Appendix 14 and 15 have a list of proficiency chemicals.
Kojima:	Are there any other comments?
Xingchao:	I agree with the comments and I think the report is improved.
Lin:	(inaudible)
Aiba:	(inaudible)

van Loveren:	The applicability domain does not seem to be defined anywhere. Where is t applicability domain? All the information is there, but there is no single cle could rename 10-6 and start with a simple explanation of the applicability d According to the reviewers' suggestion, we changed the name of 10-6 to th domain and the limitation of the IL-2 Luc assay and added a simple explan applicability domain.
Kaplan:	This is a good point. We have defined a T-cell target, so we need to say that applicability domain. We have answered to Dr. van Loveren's comment.
Aiba:	OK, I will provide a clear definition of what the applicability domain is.
Kojima:	I will share the minutes of this meeting, and then Dr. Aiba and the VMT wi validation report to share with the PRP. Perhaps you can then submit your d Kayama within one month and to be created the PRP report by Dr. Kayama
Kayama:	The most important comment today is Henk's last comment.
Aiba:	I'd like to ask Dr. Kayama to summarize the PRP comments, because I alre original comments. I would like to know what I should respond to.
Kayama:	Will the PRP report be incorporated into the validation report or separately
Kojima:	Separately attached.

添付資料 9. IL-1 Luc assay Phase I validation 試験結果

The results of the Phase I study

Line25 judge		25									
LabA Tohoku				LabB AIST tsukuba				LabC AIST shikoku			
setNo.	code No.			setNo.	code No.			setNo.	code No.		
Set1	MITA103	MITA103	S	Set1	MITB402	MITB402	S	Set1	MITC704	MITC704	S
Set2	MITA203	MITA203	S	Set2	MITB501	MITB501	S	Set2	MITC803	MITC803	S
Set3	MITA304	MITA304	S	Set3	MITB605	MITB605	S	Set3	MITC902	MITC902	S
Set1	MITA101	MITA101	N	Set1	MITB404	MITB404	N	Set1	MITC701	MITC701	N
Set2	MITA205	MITA205	N	Set2	MITB505	MITB505	N	Set2	MITC802	MITC802	N
Set3	MITA305	MITA305	N	Set3	MITB603	MITB603	N	Set3	MITC905	MITC905	N
Set1	MITA104	MITA104	N	Set1	MITB403	MITB403	N	Set1	MITC705	MITC705	N
Set2	MITA202	MITA202	N	Set2	MITB502	MITB502	N	Set2	MITC805	MITC805	N
Set3	MITA303	MITA303	N	Set3	MITB601	MITB601	N	Set3	MITC901	MITC901	N
Set1	MITA105	MITA105	S	Set1	MITB401	MITB401	S	Set1	MITC702	MITC702	S
Set2	MITA204	MITA204	S	Set2	MITB503	MITB503	S	Set2	MITC801	MITC801	S
Set3	MITA301	MITA301	S	Set3	MITB602	MITB602	S	Set3	MITC904	MITC904	S
Set1	MITA102	MITA102	N	Set1	MITB405	MITB405	N	Set1	MITC703	MITC703	N
Set2	MITA201	MITA201	N	Set2	MITB504	MITB504	N	Set2	MITC804	MITC804	N
Set3	MITA302	MITA302	N	Set3	MITB604	MITB604	N	Set3	MITC903	MITC903	N

Within laboratory reproducibility: Lab A: 100% (5/5), Lab B: 100% (5/5), Lab C 100% (5/5)
Between laboratory reproducibility: 100% (5/5)

添付資料 10.　IL-1 Luc assay Phase II validation 試験結果

	LabA Tohoku		LabB Tsukuba		LabC AIST Shikoku		Between-labolatory concodance or disconcodance
Chem No.	Code No.	Judge	Code No.	Judge	Code No.	Judge	
2	MTA117	S	MIB221	S	MTC305	S	concodance
3	MTA105	N	MIB220	N	MTC301	N	concodance
4	MTA120	N	MIB203	N	MTC318	S	disconcodance
5	MTA115	N	MIB211	N	MTC307	S	disconcodance
6	MTA111	N	MIB224	N	MTC302	N	concodance
7	MTA112	N	MIB208	N	MTC312	N	concodance
8	MTA125	S	MIB214	S	MTC303	S	concodance
11	MTA110	N	MIB218	N	MTC322	N	concodance
12	MTA124	S	MIB217	S	MTC313	S	concodance
13	MTA102	N	MIB206	N	MTC317	N	concodance
14	MTA121	N	MIB205	N	MTC324	N	concodance
15	MTA116	N	MIB223	N	MTC309	N	concodance
16	MTA118	N	MIB202	S	MTC316	N	disconcodance
17	MTA108	S	MIB204	S	MTC315	S	concodance
20	MTA113	S	MIB219	S	MTC323	S	concodance
22	MTA107	S	MIB222	S	MTC314	S	concodance
23	MTA119	N	MIB201	N	MTC306	S	disconcodance
25	MTA104	N	MIB210	N	MTC311	N	concodance
26	MTA114	S	MIB216	S	MTC304	S	concodance
27	MTA127	N	MIB227	N	MTC327	N	concodance
	Between-labolatory concodance rate						80%（16/20）

添付資料 11. IL-1 Luc assay, IL-2 Luc assay, IL-8 Luc assay data set

Chemicals	IL-2		IL-1β		IL-8 Luc
	Judge	LOEL (ug/mL)	Judge	LOEL (ug/mL)	Judge
FK506	S	0.00	N		N
Cyclosporine A	S	0.00	N		N
Actinomycin D	S	0.02	S	0.13	P
Digoxin	S	0.07	S	0.59	P
Colchicine	S	0.27	N		P
FR167653	S	1.30	S	0.49	N
Benzethonium chloride	S	1.63	N		P
Mercuric chloride	S	1.95	S	1.95	P
Chlorpromazine	S	1.95	S	3.91	P
Dibutyl phthalate	S	2.60	S	15.63	N
Amphoterycin B	S	2.60	S	1.17	P
2-Aminoanthracene	S	5.86	S	11.72	P
Isophorone diisocyanate	S	7.81	S	3.91	P
Formaldehyde	S	7.81	N		P
Pyrimethamine	S	7.81	N		P
Cobalt chloride	S	16.93	S	46.88	P
Cisplatin	S	16.93	S	46.88	P
Chloroquine	S	17.83	S	39.06	P
Minocycline	S	18.52	S	62.50	P
Mitomycin C	S	20.00	N		P
Hydrogen peroxide	S	23.44	S	375.00	P
Citral	S	25.00	S	4.88	P
Dexamethasone	S	41.67	S	0.98	N
Pentamidine isethionate	S	52.08	S	64.45	P
Lead(II) acetate	S	57.29	N		N
Azathioprine	S	58.48	S	41.55	N
Diesel exhaust particles	S	62.50	S	39.06	P
Sodium dodecyl sulfate	S	62.50	S	62.50	P
Dapsone	S	72.92	S	125.00	N
p-Nitroaniline	S	83.33	S	125.00	N
Nitrofurazone	S	83.33	N		P
Sulfasalazine	S	92.94	S	44.81	N
Nickel sulfate	S	104.17	S	375.00	P
Aluminum chloride	S	104.17	N		N
Chloroplatinicacid	S	250.00	S	23.44	P
Diethanolamin	S	250.00	S	333.33	P
Sodium bromate	S	500.00	S	500.00	P
Histamine	S	750.00	N		P
Isoniazid	S	1000.00	N		N
Triethanolamine	S	1333.33	S	1000.00	P
Magnesium sulfate	S	2000.00	N		N
Warfarin	N		N		N
Hydrocortisone	N		N		N
Lithium carbonate	N		N		P
2,4-Diaminotoluene	N		N		N
Dibenzopyrene	N		N		N
Cyclophosphamide	N		N		P
Ethanol	N		N		N
Methanol	N		N		N
Hexachlorobenzene	N		N		N
Trichloroethylene	N		N		N
Methotrexate	N		N		P
Rapamycin	N		N		N
Mizoribine	N		N		N
Mycophenolicacid	A	0.40	S	72.00	P
2-Mercaptobenzothiazole	A	16.11	S	93.75	P
Ribavirin	A	26.04	S	750.00	N
Acetaminophen	A	100.00	N		N
Nicotinamide	A	288.07	N		N
Dimethyl sulfoxide	A	2000.00	N		N

AFC antibody forming cell, *CSM* cell surface marer, *NK* natural killer cell activity, *LOEL* lowest observed effect level

添付資料 12．IL-2 Luc assay Phase I，Phase II 化学物質の免疫毒性データーベース

Chemical name	Immunotoxicity classification		NTP data					
			In vivo	*Ex vivo*		*In vitro*		Mode of action
	Classification	Rationale	immune sytem organ weight	cytokine production	TDAR	cytokine production	T cell proliferation	
Phase I study								
Dibutyl phthalate	TTC	3), 4)	**A (spleen)**			**S (IL-2, 4, IFN-g)(H) A (IL-1b)(H) x 3 S (IL-1b)**		This compound then is proposed to modulate cytokine secretion from both monocytes/macrophages and T cells.
Hydrocortisone	TTC	1)	**S (thymus) x 2 S (spleen)**		**N**	**S (IFN-a)**		
Lead(II) acetate	TTC	1)	**A(thymus)**		**S N**	**S (IFN-g, IL-1b)(H) A (IL-4)(H)**	**S(H)**	
Nickel(II) sulfate	TTC	1)	**N S (thymus)**		**N**	**A (IL-4, IFN-g)(H) S (IL-2) S (IFN-g)**		
dimethyldithiocarbamate (DMDTC)	NTTC					**S (IL-1b)**	**N(H)**	
Phase II study								
2.4-diaminotoluene	NTTC		**N (spleen) A (spleen)**		**S**	-	-	
Benzo(a)pyrene	TTC	2), 3)		**S(IL-2)**	**S x 5 A**	**A (IL-4)(H) N (IFNγ)(H) N (IL-2)(H) S (IL-2, 4, IFN-g)**	**S (H) x 2 S x 6**	Disruption of T-cell activities has been associated with B(a)P induced immunotoxic effects (Urso et al. 1986).
Cadmium Chloride	TTC	2), 3)	**A (spleen) S (spleen)**	**A (IL-2) N (IFN-γ)**	**S x 4**	**A (IFN-g)(H) S (IL-2, IFN-g) A (IFN-g) S (IL-2) A (IL-2)**	**S**	
Dibromoacetic acid (DBAA)	TTC	1), 4)	**A (spleen) S (thymus) x 2**		**N**	**S (IL-2, 4)**	**S**	Overall, studies suggest that DBAA produces immunotoxic effects through modulation of T-cell mediated cell immunity. T-cell apoptosis, through extrinsic and intrinsic pathways, are proposed to play a role in the mode of action.
Diethylstilbestrol (DES)	TTC	1), 2), 4)	**S (thymus) x 4 A (thymus) x 2 A (spleen)**	**A (IFN-g) x 3**	**S**	**A (IL-1) A (IL-2)**		DES exposure was associated with down-regulation of gene expression in the TCR complex, and the TCR and CD28 signaling pathways.
Diphenylhydantoin	TTC	2), 3), 4)		**A (IL-4) S (IFN-γ, IL-2) S (IL-1α) N (IL-6, 12)**	**S A x 2**	-	-	DPH treatment can lead to a decrease of suppressor T cells
Ethylene Dibromide (EDB)	TTC	1)	**S (thymus) S (spleen) N**		**A**	-	**S**	
Glycidol	NTTC		**N**		**S**	-	-	Studies suggest that glycidol modulates B-cell function, and NK cell and macrophage activities.111 and decreased cytotoxic T cell activity
Indomethacin	TTC	3), 4)	**N A (spleen)**		**S x 3 A x 1**	**A (IL-2)(H) A (IFN-g)(H)**	**A (H) x 4 S A x 3**	indomethacin inhibition of prostaglandin synthesis leads to altered T-cell function,
Isonicotinic Acid Hydrazide (IAH)	TTC	2)	**N x 2**			**S (IL-2)(H) A (IL-2)(H) S (IL-1)(H)**	**S (H) x 3 A (H) x 6 A N**	
Nitrobenzene	Undetermined		**A (spleen) x 3 A (thymus) x 2**		**S N**	-		effects on T-cell function may play a role in increased susceptibility to L. monocytogenes (Burns et al. 1994).
Urethane, Ethyl carbamate	TTC	1)	**S (thymus) x2 S (spleen) x 2 N A (thymus) A (spleen)**	**N (IL-2)**	**S x 2 N**	**N (IL-2, 4, IFN-g)(H) A (IFN-g)(H) S (IFN-g)(H)**	**N x 2**	
Tributyltin Chloride (TBTC)	TTC	1)	**S (thymus) x4 S (spleen) x 3**		**N S**	**A (INF-g)(H) N (IL-2, 4)(H) S (IFN-g)(H)**	**S (H) S x 3**	
Perflouorooctanoic Acid (PFOA)	TTC	1)	**S (thymus) x2 S (spleen) x 2**	**N (IFN-g)**		**S (IL-4)(H) N (IL-2)(H)**	**A (H) S (H) N (H)**	Direct modulation of NF-kB has been implicated in modulation of cytokine production and secretion (Corsini et al. 2012).
Dichloroacetic Acid (DCAA)	TTC	2), 3)	**A(spleen)**	**N (IL-2) A (IFN-γ) x 3 S (IL-4) x 2 S (IL-2)**	**N**	**A (IL-2)(H) A (IL-2, IFN-g)**		T-cell activation was one proposed mode of action for DCAA.
Toluene	NTTC		**N**		**N**		**N**	
Acetonitrile	NTTC		**S(thymus)**		**S S**	-	-	
Mannnitol	NTTC						**N (H)**	
Vanadium Pentoxide	NTTC		**N A (spleen)**			**N**	**N**	
o-Benzyl-p-chlorophenol (BCP)	NTTC		**N**		**N**	-	-	

S: Suppression, A: Augumentation, N: No effect, (H) humana study,
#: The criterion number used to define immunotoxicity

Appendix 8 Table. The summary of immunotoxicological data of 25 chemicals (continue)

The data collected by the VMT												
Chemical name	*In vitro* effect on IL-2				*In vitro* effect on IFN-γ				*In vitro* effect on IL-4			
	Effect	Animal	*in vitro (method)*	References	Effect	Animal	*in vitro (method)*	References	Effect	Animal	*in vitro (method)*	References
Phase I study												
Dibutyl phthalate					S	human	T cells (in vitro)	Hansen et al. 2015	S	human	T cells (in vitro)	Hansen et al. 2015 (0.0278~27.8 ug/mL)
Hydrocortisone	S S	human human	lymphocyte (in vitro) PBL (in vitro)	Chikanza and Panayi 1993 Goodwin et al. 1986								
Lead(II) acetate					S no effect S	mice mice human	splenocyte (ex vivo) cell line (EL-4) PBMC	Fernandez-Cabezudo et al. 2007 Wagner et al. 2006 Hemdan et al. 2005	A no effect A A	mice mice human rat	splenocyte (ex vivo) cell line (EL-4) PBMC (in vitro) ?	Fernandez-Cabezudo et al. 2007 Wagner et al. 2006 Hemdan et al. 2005 Chen et al. 2004
Nickel(II) sulfate					A A (NiCl2) A A	mice mice human rat	spleen cell (in vitro) cell line (EL-4) PBMC (in vitro) lymphoid lung cell (ex vivo)	Kim et al. 2009 Wagner et al. 2006 Thomas et al. 2003 Goutet et al. 2000	A, S A (NiCl2) A	mice mice human	spleen cell (in vitro) cell line (EL-4) PBMC (in vitro)	Kim et al. 2009 Wagner et al. 2006 Thomas et al. 2003
dimethyldithiocarbamate (DMDTC)												
Phase II study												
2.4-diaminotoluene												
Benzo(a)pyrene												
Cadmium Chloride					N (ex vivo), A (in vitro) S S (IC50=7.05E-05 M) S	rat rat human mice	splenocyte (ex vivo, in vitro) spleen cell (ex vivo) PBMC (in vitro) thymocyte, splenocyte (in vitro)	Wang et al. 2017 Demenesku et al. 2014 Kooijiman et al. 2010 Pathak and Khandelwal 2008	no effect	rat	spleen cell (ex vivo)	Demenesku et al. 2014
Dibromoacetic acid (DBAA)												
Diethylstilbestrol (DES)												
Diphenylhydantoin												
Ethylene Dibromide (EDB)												
Glycidol												
Indomethacin												
Isonicotinic Acid Hydrazide (IAH)	A	human	PBMC (in vitro), cell line (Jurkat)	Tsuboi et al. 1995								
Nitrobenzene												
Urethane, Ethyl carbamate												
Tributyltin Chloride (TBTC)					no effect (TBTO)	mice	cell line (EL-4)	Ringerike et al. 2005				
Perflouorooctanoic Acid (PFOA)												
Dichloroacetic Acid (DCAA)												
Toluene												
Acetonitrile												
Mannnitol												
Vanadium Pentoxide												
o-Benzyl-p-chlorophenol (BCP)												

S: Suppression, A: Augumentation, N: No effect, (H) humana study,
#: The criterion number used to define immunotoxicity

引用文献の記されていないデータはNTPの好意により作成して頂いた免疫毒性データベースに基づいている（昨年度の成果報告書に記載）。引用文献が書かれている文献は以下の通りである。

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添付資料 13. IL-2 data set 化学物質の免疫毒性データベース

Appendix 9 Table . The summary of immunotoxicological data of 60 chemicals in the IL-2 Luc assay data set.

Chemical name	Immunotoxicity classification		Thymus weight			Ex Vivo effect on IL-2			
	Classification	Rationale[#]	Weight	Animal	Reference	Effect	Animal	*ex vivo (method)*	Reference
FK506	TTC	1,3	decrease decrease	rat rat	Nalesnik et al. 1987 Takai et al. 1990				
Cyclosporine A	TTC	1,3	decrease no effect decrease decrease	mice mice rat mice	Auli et al. 2012 Kanariou et al. 1989 Beschorner et al. 1987 Hattori et al. 1987				
Actinomycin D	TTC	3							
Digoxin	TTC	2, 3							
Colchicine	TTC	2,3				A	human	PBMC (ex vivo)	Freed et al. 1989
FR167653	Undetermined	2, 3							
Benzethonium chloride	Undetermined	1	decrease	rat, mice	National Toxicology Program 1995				
Mercuric chloride	TTC	1,3	decrease	mice	Dieter et al. 1983				
Chlorpromazine	TTC	1,3	decrease decrease	mice rat	Auli et al. 2012 Silvestrini et al. 1967				
Amphotericin B	Undetermined	1	decrease	mice	Blanke et al. 1977				
Dibutyl phthalate	TTC	3	no effect no effect	rat rat	Zhang et al. 2013 Salazar et al. 2004				
2-Aminoanthracene	Undetermined								
Formaldehyde	TTC	2,3	no effect	rat	Vargova et al. 1993				
Pyrimethamine	Undetermined								
Isophorone diisocyanate	Undetermined								
Cisplatin	TTC	1,2,3	decrease decrease	mice mice	Kouchi et al. 1996 Sugiyama et al. 1995	S	mice	Spleen cell (ex vivo)	Kim et al. 2019
Cobalt chloride	TTC	1, 3	decrease	rat	Chetty et al. 1979				
Chloroquine	TTC	1,3	decrease	human	Garly et al. 2008				
Minocycline	TTC	3							
Mitomycin C	Undetermined								
Hydrogen peroxide	TTC	3							

S: Suppression, A: Augumentation, N: No effect, (H) humana study,

#: The criterion number used to define immunotoxicity

Appendix 9 Table . The summary of immunotoxicological data of 60 chemicals in the IL-2 Luc assay data set.(continue)

Chemical name	In vitro effect on IL-2				In vitro effeon on IFN-γ			
	Effect	**Animal**	***in vitro (method)***	**Reference**	**Effect**	**Animal**	***in vitro (method)***	**Reference**
FK506	S S S S	mice rat human human	cell line (EL-4) primary astrocyte cell (in vitro) cell line (Jurkat, Hut-78) PBMC	Wagner et al. 2006 Gabryel et al. 2004 Henderson et al. 1991 Yoshimura et al. 1989	S	mice	cell line (EL-4)	Wagner et al. 2006
Cyclosporine A	S S S S	mice mice rat human	cell line (3A9 Tcell hybridoma) cell line (EL-4) primary astrocyte cell (in vitro) cell line (Jurkat, Hut-78)	Lehmann and Williams 2018 Ringerike et al. 2005 Gabryel et al. 2004 Henderson et al. 1991	IC50=5.00E-08 M S S	human mice mice	PBMC (in vitro) cell line (EL-4) cell line (EL-4)	Kooijman et al. 2010 Wagner et al. 2006 Ringerike et al. 2005
Actinomycin D	S S	mice human	cell line (EL-4) PBMC (in vitro)	Wagner et al. 2006 Wang et al. 1984	no effect	mice	cell line (EL-4)	Wagner et al. 2006
Digoxin	S no effect S	human human human	cell line (HepG2), Th17 cell, thymocytes PBMC (in vitro) PBMC (in vitro)	Karas et al. 2018, He et al. 1998 Sheikhi et al. 2007 Gentile et al. 1997	S (ex vivo), no effect (in vitro) S (IC50=4.31E-07 M)	mice human	spleen cell (ex vivo, in vitro) PBMC (in vitro)	Hinshaw et al. 2016 Kooijman et al. 2010
Colchicine	A	human	cell line (Jurkat)	Dupuis et al. 1993	N (IC50>5.00E-04 M(=200 ug/mL)) S (in vitro) A S	human mice human human	PBMC (in vitro) spleen cell (in vitro) PBMC (in vitro)	Kooijman et al. 2010 Sosroseno 2009 Tzortzaki et al. 2007 Altindag et al. 1997
FR167653	no effect no effect	mice human	cell line (EL-4) lymphocyte (in vitro)	Wagner et al. 2006 Yamamoto et al. 1996	no effect S no effect	mice mice human	cell line (EL-4) spleen cell (ex vivo) lymphocyte (in vitro)	Wagner et al. 2006 Ando et al. 2004 Yamamoto et al. 1996
Benzethonium chloride	no effect	mice	cell line (EL-4)	Wagner et al. 2006	no effect	mice	cell line (EL-4)	Wagner et al. 2006
Mercuric chloride	S no effect A	mice mice mice	plasma (in vivo) cell line (EL-4) spleen cell	Santarelli et al. 2006 Wagner et al. 2006 Hu et al. 1997	S (IC50=3.06E-06 M) A S	human mice mice	PBMC (in vitro) cell line (EL-4) cell line (EL-4)	Kooijman et al. 2010 Wagner et al. 2006 Ringerike et al. 2005
Chlorpromazine	A S S	human rat human	whole blood (in vitro) mixed glial and microglial cell cultures (in vitro) thymocytes (in vitro)	Himmerich et al. 2011 Labuzek et al. 2005 Schleuning et al. 1989	S S	human mice	thymocytes (in vitro) Spleen cell (in vitro)	Schleuning et al. 1989 Johnson et al. 1985
Amphotericin B								
Dibutyl phthalate	S	human	T cell (in vitro)	Hansen et al. 2015	S	human	T cells (in vitro)	Hansen et al. 2015
2-Aminoanthracene	A	mice	cell line (EL-4)	Wagner et al. 2006	A	mice	cell line (EL-4)	Wagner et al. 2006
Formaldehyde					S (mRNA and protein) A	human mice	T cell (in vitro) spleen cell (ex vivo)	Sasaki et al. 2009 Fujimaki et al. 2004
Pyrimethamine	A no effect (<LOEL)	mice human	cell line (EL-4) lymphocyte (in vitro)	Wagner et al. 2006 Bygbjerg et al. 1987	no effect	mice	cell line (EL-4)	Wagner et al. 2006
Isophorone diisocyanate					no effect	mice	Lymph node (ex vivo)	Selgrade et al. 2006
Cisplatin	no effect (<LOEL) A S	mice human human	cell line (EL-4) PBL (in vitro) PBL (in vitro)	Wagner et al. 2006 Riesbeck 1999 Sfikakis et al. 1996	S A	mice mice	Spleen cell (ex vivo) cell line (EL-4)	Kim et al. 2019 Wagner et al. 2006
Cobalt chloride	S	mice	cell line (EL-4)	Wagner et al. 2006	A	mice	cell line (EL-4)	Wagner et al. 2006
Chloroquine	S	human	Synovial T cell clones	Landewe et al. 1995	A S	mice human	? (ex vivo) T cell clone	Rosa et al. 1999 Landewe et al. 1992
Minocycline	S S	human human	PBMC (in vitro) T cell clones (in vitro)	Maeda et al. 2010 Kloppenburg et al. 1995	no effect S	mice human	splenocyte (ex vivo) T cell clones (in vitro)	Chen et al. 2010 Kloppenburg et al. 1995
Mitomycin C	no effect (<LOEL) S	mice human	cell line (EL-4) mononuclear leukocyte (in vitro)	Wagner et al. 2006 Roche et al. 1988	no effect	mice	cell line (EL-4)	Wagner et al. 2006
Hydrogen peroxide	A S	mice human	cell line (EL-4) PBMC (in vitro)	Wagner et al. 2006 Freed et al. 1987	A	mice	cell line (EL-4)	Wagner et al. 2006

S: Suppression, A: Augumentation, N: No effect, (H) humana study,

#: The criterion number used to define immunotoxicity

Appendix 9 Table . The summary of immunotoxicological data of 60 chemicals in the IL-2 Luc assay data set.(continue)

Chemical name	In vitro effect on IL-4			
	Effect	Animal	*in vitro (method)*	Reference
FK506	S	mice	cell line (EL-4)	Wagner et al. 2006
Cyclosporine A	S S S	mice mice human	cell line (EL-4) cell line (EL-4) cell line (D10.G4.1)	Wagner et al. 2006 Ringerike et al. 2005 Schmidt et al. 1994
Actinomycin D	A	mice	cell line (EL-4)	Wagner et al. 2006
Digoxin				
Colchicine	A (in vitro)	mice	spleen cell (in vitro)	Sosroseno 2009
FR167653	S no effect	mice mice	cell line (EL-4) spleen cell (ex vivo)	Wagner et al. 2006 Ando et al. 2004
Benzethonium chloride	A	mice	cell line (EL-4)	Wagner et al. 2006
Mercuric chloride	A	mice	cell line (EL-4)	Wagner et al. 2006
Chlorpromazine	S A	mice human	splenic lymphocyte (in vitro) whole blood (in vitro)	Pei et al. 2014 Himmerich et al. 2011
Amphotericin B				
Dibutyl phthalate	S	human	T cells (in vitro)	Hansen et al. 2015
2-Aminoanthracene	A	mice	cell line (EL-4)	Wagner et al. 2006
Formaldehyde	no effect	human	T cell (in vitro)	Sasaki et al. 2009
Pyrimethamine	A	mice	cell line (EL-4)	Wagner et al. 2006
Isophorone diisocyanate				
Cisplatin	A no effect	mice mice	Spleen cell (ex vivo) cell line (EL-4)	Kim et al. 2019 Wagner et al. 2006
Cobalt chloride	A	mice	cell line (EL-4)	Wagner et al. 2006
Chloroquine	no effect	mice	? (ex vivo)	Rosa et al. 1999
Minocycline	no effect	mice	splenocyte (ex vivo)	Chen et al. 2010
Mitomycin C	no effect	mice	cell line (EL-4)	Wagner et al. 2006
Hydrogen peroxide	A	mice	cell line (EL-4)	Wagner et al. 2006

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#: The criterion number used to define immunotoxicity

Appendix 9 Table . The summary of immunotoxicological data of 60 chemicals in the IL-2 Luc assay data set.

Chemical name	Immunotoxicity classification		Thymus weight			Ex Vivo effect on IL-2			
	Classification	Rationale#	Weight	Animal	Reference	Effect	Animal	*ex vivo (method)*	Reference
Citral	Undetermined	1	decrease decrease	rat rat, mice	Ress et al. 2003 National Toxicology Program 2003				
Dexamethasone	TTC	1,3	decrease decrease decrease	mice mice rat	Auli et al. 2012 Munson et al. 1982 Exon et al. 1986				
Pentamidine isethionate	TTC	3							
Lead(II)acetate	TTC	1, 3	increase	rat	Bunn et al. 2001	no effect no effect	rat rat	spleen cell (ex vivo) spleen cell (ex vivo)	Bunn et al. 2001 Miller et al. 1998
Azathioprine	TTC	1,2, 3	decrease decrease	rat rat	De Waal et al. 1995 Vos and Van Loveren 1994	S S	mice, rat human	lymphocyte, thymocyte (in vitro, ex vivo) PBMC (ex vivo)	Meredith and Scott 1994 Dupont et al. 1985
Diesel exhaust particle	TTC	1, 3	decrease	rat	Tsukue et al. 2001				
Sodium dodecyl sulfate	TTC	3							
Dapsone	TTC	3	No Effect	mice	https://ntp.niehs.nih.gov/testing/types/imm/abstracts/imm90015/index.html				
Nitrofurazone	NTTC		No Effect	mice	https://ntp.niehs.nih.gov/testing/types/imm/abstracts/imm90011/index.html				
p-Nitroaniline	TTC	1,3	increase, decrease	mice	National Toxicology Program 1993b				
Sulfasalazine	TTC	1,3	decrease	rat	National Toxicology Program 1997				
Aluminium chloride	TTC	1,3	diminished thymic cellularity	mice	Synzynys et al. 2004				
Nickel sulfate	TTC	1, 3	no effect decrease decrease	mice rat rat, mice	Knight et al. 1991 Haley et al. 1990 National Toxicology Program 1996				
Hydrocortisone	TTC	1,3	decrease decrease (PND 21), increase (PND 42)	mice rat	Van Dijk et al. 1979 El Fouhil et al. 1993a, El Fouhil et al.1993b, El Fouhil and Turkall 1993				
Diethanolamine	Undetermined	1	decrease	mice	https://ntp.niehs.nih.gov/testing/types/imm/abstracts/imm20004/imm20004.html				
Chloroplatinic acid	Undetermined				X				
Sodium bromate	Undetermined	1	No Effect	mice	https://ntp.niehs.nih.gov/testing/types/imm/abstracts/imm98004/index.html				
S: Suppression, A: Augumentation, N: No effect, (H) humana study,									
#: The criterion number used to define immunotoxicity									

Appendix 9 Table . The summary of immunotoxicological data of 60 chemicals in the IL-2 Luc assay data set.(continue)

Chemical name	In vitro effect on IL-2				In vitro effeon on IFN-γ			
	Effect	**Animal**	***in vitro (method)***	**Reference**	**Effect**	**Animal**	***in vitro (method)***	**Reference**
Citral								X
Dexamethasone	S no effect S	mice mice human	cell line (3A9 Tcell hybridoma) cell line (EL-4) CBMC, PBMC (in vitro)	Lehmann and Williams 2018 Wagner et al. 2006 Bessler et al. 1996	S S S S no effect	human human mice mice mice	PBL (in vitro) T cell (in vitro) T cell clone (in vitro) splenocyte (ex vivo) cell line (EL-4)	Arya et al. 1984 Reen and Yeh 1984 Kelso and Munck 1984 Kunicka et al. 1993 Wagner et al. 2006
Pentamidine isethionate	S no effect no effect (<LOEL)	mice mice human	cell line (EL-4) cell line (EL-4) whole blood (in vitro)	Ringerike et al. 2005 Wagner et al. 2006 Van Wauwe et al. 1996	A S	mice mice	cell line (EL-4) cell line (EL-4)	Wagner et al. 2006 Ringerike et al. 2005
Lead(II)acetate	S	mice	cell line (EL-4)	Wagner et al. 2006	S no effect S	mice mice human	splenocyte (ex vivo) cell line (EL-4) PBMC	Fernandez-Cabezudo et al. 2007 Wagner et al. 2006 Hemdan et al. 2005
Azathioprine	S S	mice mice, rat	cell line (3A9 Tcell hybridoma) lymphocyte, thymocyte (in vitro, ex vivo)	Lehmann and Williams 2018 Meredith et al. 1994	S S	human human	PBMC (ex vivo) PBMC (ex vivo)	Weimar et al. 1995 Dupont et al. 1985
Diesel exhaust particle	A	mice	cell line (EL-4)	Wagner et al. 2011	S	human	T cell (in vitro)	Sasaki et al. 2009
Sodium dodecyl sulfate	S	mice	cell line (EL-4)	Ringerike et al. 2005	S (IC50=1.61E-04 M)	human mice	PBMC (in vitro) cell line (EL-4)	Kooijman et al. 2010 Ringerike et al. 2005
Dapsone	S, A S	mice mice	cell line (EL-4) splenocyte (in vitro)	Wagner et al. 2006 Peterson et al. 1997	S, A	mice	cell line (EL-4)	Wagner et al. 2006
Nitrofurazone	A	mice	cell line (EL-4)	Wagner et al. 2006	no effect	mice	cell line (EL-4)	Wagner et al. 2006
p-Nitroaniline	A	mice	cell line (EL-4)	Wagner et al. 2006	A	mice	cell line (EL-4)	Wagner et al. 2006
Sulfasalazine	S	mice	splenocyte (in vitro)	Fujiwara et al. 1990	S A	human rat	BAL cell (in vitro) CNS (in vivo)	Dobis et al. 2010 Correale et al. 1991
Aluminium chloride	S	rat	lymphocyte (in vitro)	She et al. 2012				
Nickel sulfate	S ($NiCl_2$) A A ($NiCl_2$)	human mice mice	Cell line (Jurkat) spleen cell (in vitro) cell line (EL-4)	Saito et al. 2011 Kim et al. 2009 Wagner et al. 2006	A A (NiCl2) A A	mice mice human rat	spleen cell (in vitro) cell line (EL-4) PBMC (in vitro) lymphoid lung cell (ex vivo)	Kim et al. 2009 Wagner et al. 2006 Thomas et al. 2003 Goutet et al. 2000
Hydrocortisone	S S S S	human human human human	lymphocyte (in vitro) PBL (in vitro) lymphocyte (in vitro) PBMC (in vitro)	Chikanza and Panayi 1993 Goodwin et al. 1986 Palacios and Sugawara 1982 Northoff et al. 1980				
Diethanolamine				X				
Chloroplatinic acid				X				
Sodium bromate				X				

S: Suppression, A: Augumentation, N: No effect, (H) humana study,

#: The criterion number used to define immunotoxicity

Appendix 9 Table . The summary of immunotoxicological data of 60 chemicals in the IL-2 Luc assay data set.(continue)

Chemical name	In vitro effect on IL-4					
	Effect	Animal	*in vitro (method)*	Reference		
Citral				X		
Dexamethasone	A S S	mice human mice	cell line (EL-4) cell line (D10.G4.1) splenocyte (ex vivo)	Wagner et al. 2006 Schmidt et al. 1994 Kunicka et al. 1993		
Pentamidine isethionate	A S	mice mice	cell line (EL-4) cell line (EL-4)	Wagner et al. 2006 Ringerike et al. 2005		
Lead(II)acetate	A no effect A A	mice mice human rat	splenocyte (ex vivo) cell line (EL-4) PBMC (in vitro) ?	Fernandez-Cabezudo et al. 2007 Wagner et al. 2006 Hemdan et al. 2005 Chen et al. 2004		
Azathioprine						
Diesel exhaust particle	no effect	human	T cell (in vitro)	Sasaki et al. 2009		
Sodium dodecyl sulfate						
Dapsone	S	mice	cell line (EL-4)	Wagner et al. 2006		
Nitrofurazone	no effect	mice	cell line (EL-4)	Wagner et al. 2006		
p-Nitroaniline	A	mice	cell line (EL-4)	Wagner et al. 2006		
Sulfasalazine	S	mice	mesangial cell (in vitro)	Tsai et al. 2000		
Aluminium chloride						
Nickel sulfate	A, S A (NiCl2) A	mice mice human	spleen cell (in vitro) cell line (EL-4) PBMC (in vitro)	Kim et al. 2009 Wagner et al. 2006 Thomas et al. 2003		
Hydrocortisone						
Diethanolamine						
Chloroplatinic acid						
Sodium bromate						

S: Suppression, A: Augumentation, N: No effect, (H) humana study,

#: The criterion number used to define immunotoxicity

Appendix 9 Table . The summary of immunotoxicological data of 60 chemicals in the IL-2 Luc assay data set.(continue)

Chemical name	Immunotoxicity classification		Thymus weight			Ex Vivo effect on IL-2			
	Classification	Rationale[#]	Weight	Animal	Reference	Effect	Animal	*ex vivo (method)*	Reference
Histamine	TTC	3							
Isoniazid	NTTC	1	No Effect	mice	https://ntp.niehs.nih.gov/testing/types/imm/abstracts/imm96002/index.html				
Triethanolamine	Undetermined								
Magnesium sulfate	Undetermined								
Rapamycin	TTC	1, 3	decrease	rat	Lu et al. 2015				
Mizoribine	Undetermined								
Warfarin	TTC	3							
2,4-Diaminotoluene	NTTC	1	No Effect	mice	https://ntp.niehs.nih.gov/testing/types/imm/abstracts/imm87034/index.html				
Cyclophosphamide	TTC	1	decrease decrease decrease	mice mice rat	Auli et al. 2012 https://ntp.niehs.nih.gov/testing/types/imm/abstracts/imm90015/index.html Exon et al. 1986	S	mice	splenocyte (ex vivo)	Tabi et al. 1988
Dibenzopyrene	Undetermined	3							
Ethanol	TTC	1, 3	decrease	mice	Kim and Park 2002				
Hexachlorobenzene	Undetermined	1,2	no effect decrease cortical atrophy	rat mice monkey	Vos et al. 1979 Loose et al. 1978 Iatropoulos et al. 1976	A A	rat rat	spleen cell (ex vivo) spleen cell (ex vivo)	Ezendam et al. 2004 Vandebriel et al. 1998
Lithium carbonate	TTC	1,3	decrease	mice	https://ntp.niehs.nih.gov/testing/types/imm/abstracts/imm85001/index.html				
Methanol	NTTC	1	decrease	rat	Parthasarathy et al. 2005				
Methotrexate	TTC	3							
Dimethyl sulfoxide	NTTC	1,3	no effect	mice	Caren et al. 1985				

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#: The criterion number used to define immunotoxicity

Appendix 9 Table . The summary of immunotoxicological data of 60 chemicals in the IL-2 Luc assay data set.(continue)

Chemical name	In vitro effect on IL-2				In vitro effeon on IFN-γ			
	Effect	**Animal**	***in vitro (method)***	**Reference**	**Effect**	**Animal**	***in vitro (method)***	**Reference**
Histamine	S S A, S	mice human mice	splenocyte (in vitro) PBMC (in vitro) spleen cell (in vitro)	Poluektova et al. 1999 Huchet and Grandjon 1988 Khan et al. 1985	no effect	mice	serum (in vivo)	Metushi and Uetrecht 2014
Isoniazid	S (13.7, 137.1 ug/mL), A (0.0137~1.37 ug/mL)	human	T cell (in vitro)	Kucharz and Sierakowski 1990				
Triethanolamine				X				
Magnesium sulfate								
Rapamycin	A, S A (0.0009ug/mL), S (0.457ug/mL) S S	mice rat human human	cell line (EL-4) primary astrocyte cell (in vitro) T cell (in vitro) cell line (Jurkat, Hut-78)	Ringerike et al. 2005 Gabryel et al. 2004 Hanke et al. 1992 Henderson et al. 1991	no effect	mice	cell line (EL-4)	Ringerike et al. 2005
Mizoribine	S (>LOEL) no effect	mice human	T cells (in vitro) peripheral blood T cells (in vitro)	Song et al. 2006 Turka et al. 1991				
Warfarin	S	human	T cell (in vitro)	Bruserud and Lundin 1	S (IC50=3.16E-04 M)	human	PBMC (in vitro)	Kooijman et al. 2010
2,4-Diaminotoluene				X X				X
Cyclophosphamide	no effect (needs metabolization)	mice	cell line (3A9 Tcell hybridoma)	Lehmann and Williams 2018				
Dibenzopyrene	A	mice	cell line (EL-4)	Wagner et al. 2006	A	mice	cell line (EL-4)	Wagner et al. 2006
Ethanol	S	human	cell line (Jurkat), primary CD4+ T lymphocytes (in vitro)	Ghare et al. 2011	N (IC50>1.00E-03 M)	human	PBMC (in vitro)	Kooijman et al. 2010
Hexachlorobenzene					N (IC50>1.00E-05 M)	human	PBMC (in vitro)	Kooijman et al. 2010
Lithium carbonate	A A A	human human human	PBMC (in vitro) PBMC (in vitro) PBMC (in vitro)	Wilson et al. 1989 Parenti et al. 1988 Sztein et al. 1987	N (IC50>1.00E-03 M)	human	PBMC (in vitro)	Kooijman et al. 2010
Methanol	no effect	mice	cell line (EL-4)	Wagner et al. 2006	N (IC50>1.00E-03 M) no effect	human mice	PBMC (in vitro) cell line (EL-4)	Kooijman et al. 2010 Wagner et al. 2006
Methotrexate	S A	mice human	cell line (3A9 Tcell hybridoma) PBMC (in vitro)	Lehmann and Williams 2018 Cesario et al. 1984				
Dimethyl sulfoxide	S, A no effect (1 %), S (2.5, 5, 10 %)	mice human	cell line (EL-4) PBMC (in vitro)	Wagner et al. 2006 de Abreu Costa et al. 2017	no effect	mice	cell line (EL-4)	Wagner et al. 2006

S: Suppression, A: Augumentation, N: No effect, (H) humana study,

#: The criterion number used to define immunotoxicity

Appendix 9 Table . The summary of immunotoxicological data of 60 chemicals in the IL-2 Luc assay data set.(continue)

Chemical name	In vitro effect on IL-4					
	Effect	Animal	*in vitro (method)*	Reference		
Histamine						
Isoniazid						
Triethanolamine						
Magnesium sulfate						
Rapamycin	S	mice	cell line (EL-4)	Ringerike et al. 2005		
Mizoribine						
Warfarin						
2,4-Diaminotoluene				X		
Cyclophosphamide						
Dibenzopyrene	A	mice	cell line (EL-4)	Wagner et al. 2006		
Ethanol						
Hexachlorobenzene						
Lithium carbonate						
Methanol	A	mice	cell line (EL-4)	Wagner et al. 2006		
Methotrexate	no effect	human	cell line (D10.G4.1)	Schmidt et al. 1994		
Dimethyl sulfoxide	A	mice	cell line (EL-4)	Wagner et al. 2006		

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#: The criterion number used to define immunotoxicity

Appendix 9 Table . The summary of immunotoxicological data of 60 chemicals in the IL-2 Luc assay data set.(continue)

Chemical name	Immunotoxicity classification		Thymus weight			Ex Vivo effect on IL-2			
	Classification	Rationale#	Weight	Animal	Reference	Effect	Animal	*ex vivo (method)*	Reference
Trichloroethylene	NTTC	1	No Effect	mice, rat	https://ntp.niehs.nih.gov/testing/types/imm/abstracts/imm20006/imm20006.html https://ntp.niehs.nih.gov/testing/types/imm/abstracts/imm96007/imm96007.html				
Mycophenolic acid	Undetermined	1, 3	decrease	rat	Pally et al. 2001				
2-Mercaptobenzothiazole	Undetermined								
Ribavirin	TTC	1, 3	decrease	mice	https://ntp.niehs.nih.gov/testing/types/imm/abstracts/imm90010/index.html				
Nicotinamide	Undetermined								
Acetaminophen	Undetermined		no effect decrease (rat), no effect (mice)	mice rat, mice	Kim and Park 2002 National Toxicology Program 1993a				

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#: The criterion number used to define immunotoxicity

Appendix 9 Table . The summary of immunotoxicological data of 60 chemicals in the IL-2 Luc assay data set.(continue)

Chemical name	In vitro effect on IL-2				In vitro effeon on IFN-γ			
	Effect	**Animal**	***in vitro (method)***	**Reference**	**Effect**	**Animal**	***in vitro (method)***	**Reference**
Trichloroethylene								
Mycophenolic acid	no effect no effect	human mice	PBL (in vitro) spleen cell (in vitro)	Quemeneur et al. 2002 Lemster et al. 1992				
2-Mercaptobenzothiazole								
Ribavirin	A A	human human	PBMC (in vitro) T cells (in vitro)	Sookoian et al. 2004 Tam et al. 1999				
Nicotinamide								
Acetaminophen	A	mice	cell line (EL-4)	Wagner et al. 2006	A N (C50>5.00E-04 M)	mice human	cell line (EL-4) PBMC (in vitro)	Wagner et al. 2006 Kooijman et al. 2010

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#: The criterion number used to define immunotoxicity

Appendix 9 Table . The summary of immunotoxicological data of 60 chemicals in the IL-2 Luc assay data set.(continue)

Chemical name	In vitro effect on IL-4					
	Effect	**Animal**	***in vitro (method)***	**Reference**		
Trichloroethylene						
Mycophenolic acid						
2-Mercaptobenzothiazole						
Ribavirin						
Nicotinamide						
Acetaminophen	A	mice	cell line (EL-4)	Wagner et al. 2006		

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#: The criterion number used to define immunotoxicity

Chemical name	Immunotoxicity classification		NTP data					
			In vivo	*Ex vivo*		*In vitro*		
	Classification	Rationale	immune sytem organ weight	cytokine production	TDAR	cytokine production	T cell proliferation	Mode of action
Phase I study								
Dibutyl phthalate	TTC	3), 4)	**A (spleen)**			**S (IL-2, 4, IFN-g)(H) A (IL-1b)(H) x 3 S (IL-1b)**		This compound then is proposed to modulate cytokine secretion from both monocytes/macrophages and T cells.
Hydrocortisone	TTC	1)	**S (thymus) x 2 S (spleen)**		**N**	**S (IFN-a)**		
Lead(II) acetate	TTC	1)	**A(thymus)**		**S N**	**S (IFN-g, IL-1b)(H) A (IL-4)(H)**	**S(H)**	
Nickel(II) sulfate	TTC	1)	**N S (thymus)**		**N**	**A (IL-4, IFN-g)(H) S (IL-2) S (IFN-g)**		
dimethyldithiocarbamate (DMDTC)	NTTC					**S (IL-1b)**	**N(H)**	
Phase II study								
2.4-diaminotoluene	NTTC		**N (spleen) A (spleen)**		**S**	-	-	
Benzo(a)pyrene	TTC	2), 3)		**S(IL-2)**	**S x 5 A**	**A (IL-4)(H) N (IFNγ)(H) N (IL-2)(H) S (IL-2, 4, IFN-g)**	**S (H) x 2 S x 6**	Disruption of T-cell activities has been associated with B(a)P induced immunotoxic effects (Urso et al. 1986).
Cadmium Chloride	TTC	2), 3)	**A (spleen) S (spleen)**	**A (IL-2) N (IFN-γ)**	**S x 4**	**A (IFN-g)(H) S (IL-2, IFN-g) A (IFN-g) S (IL-2) A (IL-2)**	**S**	
Dibromoacetic acid (DBAA)	TTC	1), 4)	**A (spleen) S (thymus) x 2**		**N**	**S (IL-2, 4)**	**S**	Overall, studies suggest that DBAA produces immunotoxic effects through modulation of T-cell mediated cell immunity. T-cell apoptosis, through extrinsic and intrinsic pathways, are proposed to play a role in the mode of action.
Diethylstilbestrol (DES)	TTC	1), 2), 4)	**S (thymus) x 4 A (thymus) x 2 A (spleen)**	**A (IFN-g) x 3**	**S**	**A (IL-1) A (IL-2)**		DES exposure was associated with down-regulation of gene expression in the TCR complex, and the TCR and CD28 signaling pathways.
Diphenylhydantoin	TTC	2), 3), 4)		**A (IL-4) S (IFN-γ, IL-2) S (IL-1α) N (IL-6, 12)**	**S A x 2**	-	-	DPH treatment can lead to a decrease of suppressor T cells
Ethylene Dibromide (EDB)	TTC	1)	**S (thymus) S (spleen) N**		**A**	-	**S**	
Glycidol	NTTC		**N**		**S**	-	-	Studies suggest that glycidol modulates B-cell function, and NK cell and macrophage activities.111 and decreased cytotoxic T cell activity
Indomethacin	TTC	3), 4)	**N A (spleen)**		**S x 3 A x 1**	**A (IL-2)(H) A (IFN-g)(H)**	**A (H) x 4 S A x 3**	indomethacin inhibition of prostaglandin synthesis leads to altered T-cell function,
Isonicotinic Acid Hydrazide (IAH)	TTC	2)	**N x 2**			**S (IL-2)(H) A (IL-2)(H) S (IL-1)(H)**	**S (H) x 3 A (H) x 6 A N**	
Nitrobenzene	Undetermined		**A (spleen) x 3 A (thymus) x 2**		**S N**	-		effects on T-cell function may play a role in increased susceptibility to L. monocytogenes (Burns et al. 1994).
Urethane, Ethyl carbamate	TTC	1)	**S (thymus) x2 S (spleen) x 2 N A (thymus) A (spleen)**	**N (IL-2)**	**S x 2 N**	**N (IL-2, 4, IFN-g)(H) A (IFN-g)(H) S (IFN-g)(H)**	**N x 2**	
Tributyltin Chloride (TBTC)	TTC	1)	**S (thymus) x4 S (spleen) x 3**		**N S**	**A (INF-g)(H) N (IL-2, 4)(H) S (IFN-g)(H)**	**S (H) S x 3**	
Perflouorooctanoic Acid (PFOA)	TTC	1)	**S (thymus) x2 S (spleen) x 2**	**N (IFN-g)**		**S (IL-4)(H) N (IL-2)(H)**	**A (H) S (H) N (H)**	Direct modulation of NF-kB has been implicated in modulation of cytokine production and secretion (Corsini et al. 2012).
Dichloroacetic Acid (DCAA)	TTC	2), 3)	**A(spleen)**	**N (IL-2) A (IFN-γ) x 3 S (IL-4) x 2 S (IL-2)**	**N**	**A (IL-2)(H) A (IL-2, IFN-g)**		T-cell activation was one proposed mode of action for DCAA.
Toluene	NTTC		**N**		**N**		**N**	
Acetonitrile	NTTC		**S(thymus)**		**S S**	-	-	
Mannnitol	NTTC						**N (H)**	
Vanadium Pentoxide	NTTC		**N A (spleen)**			**N**	**N**	
o-Benzyl-p-chlorophenol (BCP)	NTTC		**N**		**N**	-	-	

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#: The criterion number used to define immunotoxicity

Appendix 8 Table. The summary of immunotoxicological data of 25 chemicals (continue)

The data collected by the VMT												
Chemical name	*In vitro* effect on IL-2				*In vitro* effect on IFN-γ				*In vitro* effect on IIL-4			
	Effect	Animal	*in vitro (method)*	References	Effect	Animal	*in vitro (method)*	References	Effect	Animal	*in vitro (method)*	References
Phase I study												
Dibutyl phthalate					S	human	T cells (in vitro)	Hansen et al. 2015	S	human	T cells (in vitro)	Hansen et al. 2015 (0.0278−27.8 ug/mL)
Hydrocortisone	S S	human human	lymphocyte (in vitro) PBL (in vitro)	Chikanza and Panayi 1993 Goodwin et al. 1986								
Lead(II) acetate					S no effect S	mice mice human	splenocyte (ex vivo) cell line (EL-4) PBMC	Fernandez-Cabezudo et al. 2007 Wagner et al. 2006 Hemdan et al. 2005	A no effect A A	mice mice human rat	splenocyte (ex vivo) cell line (EL-4) PBMC (in vitro) ?	Fernandez-Cabezudo et al. 2007 Wagner et al. 2006 Hemdan et al. 2005 Chen et al. 2004
Nickel(II) sulfate					A A (NiCl2) A A	mice mice human rat	spleen cell (in vitro) cell line (EL-4) PBMC (in vitro) lymphoid lung cell (ex vivo)	Kim et al. 2009 Wagner et al. 2006 Thomas et al. 2003 Goutet et al. 2000	A, S A (NiCl2) A	mice mice human	spleen cell (in vitro) cell line (EL-4) PBMC (in vitro)	Kim et al. 2009 Wagner et al. 2006 Thomas et al. 2003
dimethyldithiocarbamate (DMDTC)												
Phase II study												
2.4-diaminotoluene												
Benzo(a)pyrene												
Cadmium Chloride					N (ex vivo), A (in vitro) S S (IC50=7.05E-05 M) S	rat rat human mice	splenocyte (ex vivo, in vitro) spleen cell (ex vivo) PBMC (in vitro) thymocyte, splenocyte (in vitro)	Wang et al. 2017 Demenesku et al. 2014 Kooijman et al. 2010 Pathak and Khandelwal 2008	no effect	rat	spleen cell (ex vivo)	Demenesku et al. 2014
Dibromoacetic acid (DBAA)												
Diethylstilbestrol (DES)												
Diphenylhydantoin												
Ethylene Dibromide (EDB)												
Glycidol												
Indomethacin												
Isonicotinic Acid Hydrazide (IAH)	A	human	PBMC (in vitro), cell line (Jurkat)	Tsuboi et al. 1995								
Nitrobenzene												
Urethane, Ethyl carbamate												
Tributyltin Chloride (TBTC)					no effect (TBTO)	mice	cell line (EL-4)	Ringerike et al. 2005				
Perflouorooctanoic Acid (PFOA)												
Dichloroacetic Acid (DCAA)												
Toluene												
Acetonitrile												
Mannnitol												
Vanadium Pentoxide												
o-Benzyl-p-chlorophenol (BCP)												

S: Suppression, A: Augumentation, N: No effect, (H) humana study,
#: The criterion number used to define immunotoxicity

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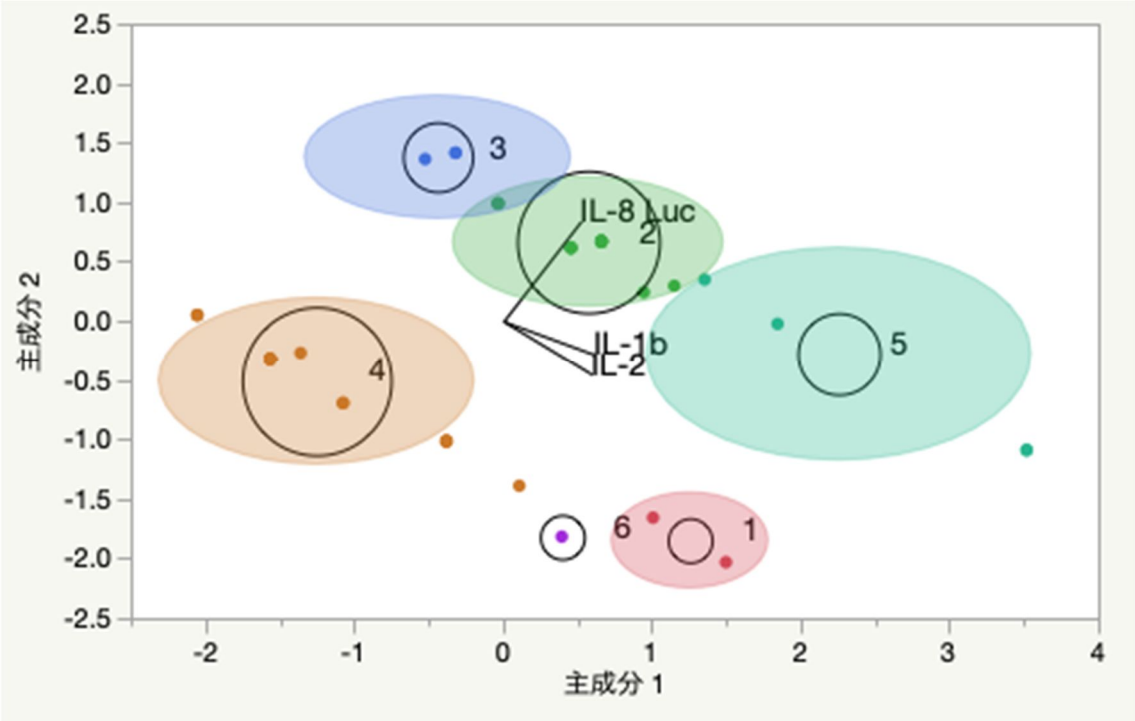
添付資料 14．MITA による化学物質の免疫毒性プロフィル



添付資料 15. Detailed review paper content.

Potential title:
"*In vitro* immunotoxicity testing"
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